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ACIDITY OF RHUBARB PETIOLES AS INFLUENCED BY STAGE OF MATURITY, SEASON AND PARTIAL SHADING[1]

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ABSTRACT

Field experiments were conducted to determine the effect of stage of maturity and season on the acidity of rhubarb petioles. Some physiological or biochemical changes with age of petioles were determined. The changes in petioles of similar age developed at different periods of the growing season were also determined. The effect of shading the plants with cheesecloth was studied. Acidity determinations indicated that the younger petioles were more acid than older ones. The soluble potassium content increased as the petioles aged. The acid content of the petioles was low at the beginning of the harvesting period but was significantly higher at the end. Petioles grown in full sunshine were more acid than those grown in partial shade.

INTRODUCTION

The common cultivated rhubarb, *Rheum rhaponticum* L., is one of the few vegetables in which the petiole or leaf-stalk is the part consumed. This succulent petiole has a characteristic tart flavour and is treated more as an acid fruit than as a vegetable, being stewed and eaten as a sauce or made into pies with a liberal addition of sugar in order to obtain an agreeable taste. In a study of the food value and cooking and canning qualities of rhubarb, Culpepper and Moon (7) found that the acid content varied considerably with the season and influenced the quantity of sugar necessary for adequate sweetening. Several other methods are usually suggested in order to reduce tartness or neutralize partially the acidity of rhubarb and obtain a milder product. Culpepper and Moon (7) mention that rhubarb is improved by soaking in warm water for a short time or in cold water for a long time. Among other suggested methods for reducing tartness (1) are: addition of sodium chloride, or soda; scalding; mixing with egg for pie, or combining rhubarb with other fruits and berries.

The proportions in which the acid and sugar occur in rhubarb seem to have an important influence on the eating quality. A number of factors may exert an influence on the quantitative composition of rhubarb and consequently on its palatability.

In the present investigation, the effects of stage of maturity and season on the acidity of rhubarb petioles were determined. The effect of shading the plants with cheesecloth was also studied. Such studies should provide

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useful data for establishing the optimum harvest period and determining the effects of the various factors on the acidity of the rhubarb petioles.

MATERIAL AND METHODS

The material used in these studies of the effects of stage of maturity, season and partial shading on the acidity of rhubarb petioles consisted of a 4-year-old planting of the Macdonald variety of rhubarb grown at the Experimental Farm, Ste. Anne de la Pocatière, Quebec. A 100-foot row was divided into two blocks of 50 feet each. The first block was left exposed to direct sunlight throughout the experiment, from April 24 to June 24, 1954, and the second block was shaded with cheesecloth.

On April 19, the leaves were appearing above ground. On April 24, they were about 2 to 3 inches high and the leaf blade was not fully expanded. These leaves were then tagged, and successively, at regular 10-day intervals, petioles about 2 to 3 inches in length were tagged.

Harvests were made 10, 20, 30 and 40 days after tagging. At each harvest, 20 petioles of all ages available were sampled for chemical analysis. The leaves were harvested in the afternoon. Each sample consisted of about 20 leaves from 15 plants. The leaf blades were discarded and the petioles were brought into the laboratory and cut into pieces about $\frac{1}{2}$-inch long. Hundred-gram samples were placed in Sealright containers and frozen immediately at $-20°$ C.

The chemical determinations were made on the frozen samples about a month after harvest. The samples were removed from the freezer and allowed to thaw at room temperature. Each sample was placed in clean cheesecloth and uniformly packed in the press cylinder of a Carver laboratory press. The juice was expressed at 10,000-lb. pressure for 10 minutes. In order to obtain as uniform a sample as possible, and to avoid plugging of the electrode in pH determinations or volume errors in titrations due to solid material, the juice was centrifuged for 10 minutes at a speed of about 1,000 r.p.m. to remove the coarse material in suspension.

The hydrogen ion concentration determinations were then made immediately on the centrifuged juice without the addition of water. A model H2 Beckman Glass Electrode pH meter, with a general purpose glass electrode and a fibre type calomel reference electrode, was used. The values are expressed as pH.

The total titratable acidity was determined electrometrically by neutralization of a 5-ml. sample of the centrifuged juice (diluted with distilled water to 50 ml.) with 0.1 N sodium hydroxide to pH 7.8. The results were converted to grams of malic acid per 100 ml. of expressed juice.

The refractive index of the centrifuged juice (total soluble solids) was determined, using an Abbé refractometer having sucrose and refractive index scales. The readings were made at 20° C., the room temperature being approximately the same.

The potassium, calcium and magnesium contents of the centrifuged juice were determined by flame photometry in a model DU Beckman Quartz Spectrophotometer, using standard solutions of these elements and

curves obtained with these standards for comparison. The calcium was determined on the undiluted sap, but the determinations of potassium and magnesium were made on 5-ml. aliquots of centrifuged sap diluted to 100 ml. with 0.2 N acetic acid.

EXPERIMENTAL RESULTS

Effect of Age on Chemical Composition of Petioles

The results of these field experiments show that the age at which rhubarb petioles should be harvested depends on the environmental conditions during which they developed. Only a small proportion of the first petioles developed by the rhubarb plants in the spring reached marketable size. Most of them died because of subsequent unfavourable weather conditions.

Petioles 20 or even 10 days old at the end of May or the beginning of June and which developed rapidly under more favourable conditions were of marketable size, whereas most of the petioles over 30 days old were still unmarketable. The optimum age for market varied from 10 to 30 days depending on environmental conditions. Petioles of the Macdonald variety over 30 days old had a poor, greenish appearance and were more fibrous and tougher than younger petioles. The colour of the expressed juice was highly correlated with the age of the petioles. The juice of younger petioles was deep red, whereas the juice of older petioles was pinkish to greenish. No petioles reached 60 days after tagging; very few ever reached 50 days after tagging, and these were unmarketable.

The results of the chemical analyses of rhubarb petioles of known ages at different sampling dates are presented in Table 1.

TABLE 1.—COMPOSITION OF RHUBARB PETIOLES OF DIFFERENT AGES

Age in days	Acidity as malic acid gm./100 ml.	pH	Soluble K p.p.m	Soluble Ca p.p.m	Soluble Mg p.p.m	Total soluble solids as sucrose
10	1.65	3.1	3330	0.8	101	3.0
20	1.73	3.2	4167	0.9	101	3.5
30	1.71	3.3	4833	1.1	101	3.6
40	1.22	3.4	5817	1.0	113	2.8
Mean	1.58	3.2	4537	0.9	104	3.2
L.S.D. (P = .05)	0.36	0.1	988	N.S.	N.S.	N.S.
L.S.D. (P = .01)	N.S.	0.2	1497	N.S.	N.S.	N.S.

TABLE 2.—COMPOSITION OF RHUBARB PETIOLES AT DIFFERENT DATES

Date of sampling	Acidity as malic acid gm./100 ml.	pH	Soluble K p.p.m	Soluble Ca p.p.m	Soluble Mg p.p.m	Total soluble solids as sucrose
May 24	1.30	3.5	4202	1.0	108	3.2
June 3	1.62	3.2	4056	1.0	100	3.2
June 13	1.79	3.1	4163	0.9	84	3.5
June 23	1.69	3.2	4110	0.9	120	3.5
Mean	1.60	3.3	4133	0.95	103	3.3
L.S.D. (P = .05)	.06	.09	N.S.	N.S.	N.S.	N.S.
L.S.D. (P = .01)	.09	.14	N.S.	N.S.	N.S.	N.S.

Table 1 shows that the differences in titratable acidity expressed as malic acid, the pH differences, and the soluble potassium content of petioles of different ages are significant. The soluble calcium and magnesium contents, as well as the total soluble solids (refractometer reading) differences between petioles of different ages were not significant.

The titratable acidity of the petioles remained constant or increased slightly until the petioles were from 20 to 30 days old and decreased rapidly thereafter. The pH increased very significantly with age. The younger petioles were more acid than the older at different dates. These pH changes are related to titratable acidity changes of petioles of increasing age.

The soluble potassium content of the petioles increased very markedly with age. The amount of soluble potassium present in the 40-day-old petioles was for some samples almost double that of the 10-day petioles.

Effect of Season on Chemical Composition of Petioles

To determine the extent of seasonal changes, petioles of the same age were compared for different sampling dates. The results of the chemical analyses of rhubarb petioles of same ages at different dates during the growth period are presented in Table 2.

Titratable acidity of petioles of the same age varied greatly throughout the period of this experiment. Early in the season, the titratable acidity was low, but it was significantly higher at the end of the harvest season.

The pH changes were closely related to titratable acidity changes throughout the season. The pH of rhubarb petioles was rather high early in the season but it declined during the season and increased slightly at the last sampling date. This is interpreted as indicating that the acidity of the petioles increased with the season, which is also shown by titratable acidity changes.

TABLE 3.—COMPOSITION OF RHUBARB PETIOLES AS INFLUENCED BY PARTIAL SHADING

Treatment	Acidity as malic acid gm./100 ml.	pH	Total soluble solids as sucrose
Unshaded	1.58	3.2	3.3
Shaded	1.34	3.2	2.7
Mean	1.46	3.2	3.0
L.S.D. (P = .05)	0.12	N.S.	0.3
L.S.D. (P = .01)	N.S.	N.S.	N.S.

The soluble potassium content of the petioles remained fairly constant during the period of the experiment. The differences between petioles of same age at different dates during the season were small and not significant. The soluble calcium and magnesium contents, as well as the refractometer reading differences between petioles of same age at different dates, were not significant.

Effect of Partial Shading on Chemical Composition of Petioles

The results of the chemical analyses of the shaded and the unshaded series petioles are presented in Table 3. The pH values, the titratable acidity and the total soluble solids content of both series of petioles were compared in order to determine the influence of partial shading.

When both series of petioles are compared, the difference in titratable acidity was significant. The petioles grown in full sunshine were more acid than those grown in partial shade. However, the pH differences between petioles of the two series were not significant. The total soluble solids content of the shaded petioles was significantly lower than that of the unshaded petioles.

DISCUSSION

The acidity determinations were made on the expressed sap of rhubarb petioles. The acidity was expressed in terms of pH and titratable acidity. These results reflected the changes which occurred in the organic acid content of the rhubarb petioles.

Earlier work (8) showed that titration is a useful means of ascertaining large changes in some of the organic constituents of the plant sap. Vickery, Pucher, Wakeman and Leavenworth (23) found that the acids in rhubarb petioles were principally malic, oxalic and citric, together with a small amount of acids of unknown nature. With the exception of oxalic acid, these acids have dissociation constants of about the same order of magnitude

and would be expected to react similarly in titration. The oxalic acid of the petioles was usually present in comparatively small amounts (17), and much of this as the insoluble calcium salt (16, 23). For these reasons, the titration procedure used probably gives a good estimate of the free acid content of the juice expressed from rhubarb petioles.

Determination of acidity in sap expressed by pressure has been criticized by Rea and Small (20) because it furnishes no very obvious clues to the process by which it is brought about. Investigations of expressed sap of various plants have not, however, proved entirely fruitless because the degree of acidity has been found to be measurably correlated with illumination (10, 11, 22), photoperiod (9), temperature (12), age (4), season (2), and nutrient conditions (5, 13, 14). Discovery of such correlations was often the initial step in the determination of their underlying physiological mechanisms.

The results of the experiments reported here can, therefore, be interpreted as a measure of the influence of the various factors upon the changes of the organic acid content of the rhubarb petioles. They indicated that the acidity of the petioles was a variable quantity, determined by age and environmental factors.

There was a seasonal pattern in the acid content of rhubarb petioles as the harvest season progressed. In general, the acid content was low at the beginning of the harvesting period but it was significantly higher at the end. The seasonal changes in acidity of rhubarb petioles were undoubtedly caused in part by environmental factors since petioles of the same age were compared at each sampling date. Other workers (3, 6, 7, 19) have also found a marked difference in the acidity of rhubarb petioles at different periods of the season.

Acidity determinations of petioles of known ages at different sampling dates indicated that the younger petioles were more acid than older ones. Conversely, the soluble potassium content of the petioles increased markedly with age. This might explain in part the lower titratable acidity values of the older petioles, since such determinations measure merely that part of the water-soluble acids that remains physiologically unneutralized. In many plants the potassium contained in the tissues is almost completely water soluble (15, 21). Potassium is frequently the chief soluble mineral cation present in the cellular fluids, particularly in plants which normally contain little calcium or in which most of the calcium is present in insoluble form (8, 18). It is generally supposed that much of the potassium present in plants is in the form of salts of organic acids and that these acids and their salts constitute the principal components of the cellular buffer systems.

The acid content of petioles from rhubarb plants growing in partial shade was lower than the acid content of petioles from plants grown in full sunshine. Steinmann (22) found also that the acidity increased in rhubarb leaves during exposure to sunlight and decreased in leaves kept in darkness. Since the source of the organic acids is known to be dependent upon sugar, directly or indirectly, factors which influence the formation of carbohydrates and sugars indirectly influence the production of these acids.

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INFLUENCE OF NITROGEN, PHOSPHORUS AND POTASSIUM ON THE COOKING QUALITY OF POTATOES[1]

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ABSTRACT

In general, the dry matter content (as indicated by specific gravity) of Katahdin potatoes grown on seven different soils in 1955 and 1956 was sometimes increased by phosphorus, slightly decreased by nitrogen, and consistently decreased by potassium chloride fertilizer applications. On the other hand, the consumer preference index (based on a sensory evaluation of the texture, flavour and colour of the cooked tubers by a panel of three judges) was increased by both phosphorus and potassium chloride applications and was variable for nitrogen. Because potato cooking quality is the summary effect of the texture, flavour and colour of the cooked product, the consumer preference index should be a better measure of this quality than the dry matter content, which refers only to the textural component. The differences, i.e. increases or decreases in dry matter content or consumer preference index due to fertilizer treatment, were small in relation to those associated with differences in soils and weather. However, chemical fertilizers may have a marked effect on the yields obtainable.

Abundant experimental evidence shows that chemical fertilizers substantially increase yields of potatoes. However, evidence concerning the effect of chemical fertilizers on the cooking quality of potatoes is less conclusive due, in part, to the difficulties involved in evaluating and defining potato cooking quality.

Specific gravity measurements of potato tubers have been used by many workers as an indication of the dry matter percentage and, by inference, the cooking quality. On the basis of such measurements, Terman and co-workers (12, 13, 14) concluded, from a study of 20 years' results in Maine, that nitrogen and phosphorus fertilizers caused minor changes in potato quality, but potassium fertilizer caused substantial reduction in quality. However, Hill (7) reported that potash fertilizers only lowered potato quality when used at rates which allowed luxury absorption.

Several workers have shown a lack of correlation between the specific gravity and the actual mealiness of cooked tubers (3, 5, 6, 11, 15). A sensory evaluation of mealiness (texture), therefore, should be a better, although perhaps less precise, measure of the texture of cooked tubers. Hester and Bennet (5) found that differences in specific gravity measurements did not account for differences in quality due to colour and flavour variations among the lots of potatoes that they tested.

In an extensive investigation into the cooking quality of potatoes: its evaluation and relationship to potato characteristics, Kirkpatrick (10) stated that cooking quality in potatoes is understood to refer to characteristics of appearance and taste which potatoes possess when cooked. For best quality, she further stated, it is generally agreed that boiled, mashed and baked potatoes should be creamy white in colour, should have a moder-

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ately dry to dry and mealy texture, and a good natural potato flavour. This concept of the characteristics desired in cooked potatoes has been substantiated by surveys conducted among household and institutional buyers (2, 8, 9). Therefore, a sensory evaluation of the summary effect of texture, flavour and colour, i.e. a consumer preference index, of the cooked tubers by a trained panel of judges (10, 11), may be the most valid method of evaluating potato quality.

The objectives of this study were: 1) to compare the consumer preference index method and the specific gravity or dry matter percentage method for evaluating the cooking quality of potatoes as influenced by different rates of applied nitrogen, phosphorus and potassium fertilizers, by different soil types and by different seasons, and 2) to determine whether high yields per acre were incompatible with good cooking quality of potatoes.

MATERIALS AND METHODS

Three levels of nitrogen, phosphorus and potassium were applied for Katahdin potatoes on three farms in 1955 and on four farms in 1956. The soil types involved were: Fox sandy loam, Guelph sandy loam, Honeywood silt loam, Bookton loam and Tioga loam (Table 1). The plots were four rows wide and 25 feet long in a confounded 3 x 3 x 3 factorial arrangement in incomplete blocks of nine treatments. Three replications were used. Appropriate statistical analyses of the experimental data were performed according to methods outlined in Cochrane and Cox (1). The soils on the experimental sites varied from very low to medium in organic matter, phosphorus and potassium content, and were neutral to slightly acid in reaction.

The exact amount of nitrogen (N) as ammonium nitrate, phosphorus (P_2O_5) as 20 per cent superphosphate, and potassium (K_2O) as potassium chloride was weighed for each row of each plot. An endless-belt type

TABLE 1.—SOIL TYPE, pH, PER CENT ORGANIC MATTER, PHOSPHORUS AND POTASSIUM SOIL TEST OF SEVEN SOILS IN 1955 AND 1956 POTATO TRIALS

Year	Soil type	pH	O.M. %	Phosphorus[1]		Potassium[2]	
				lb./acre	Rating	lb./acre	Rating
1955	Fox sandy loam	6.8	1.4	215	Low	99	Low
	Guelph sandy loam	6.2	3.0	396	Med.−	244	Med.+
	Honeywood silt loam	6.7	4.7	446	Med.−	282	High−
1956	Bookton loam	6.4	4.7	122	Low−	142	Low+
	Tioga loam	6.0	2.7	328	Low+	72	Low−
	Fox sandy loam	6.2	3.2	537	Med.	271	Med.+
	Honeywood silt loam	7.2	3.9	397	Med.−	127	Low+

[1] Soil shaken 1 minute with 0.5N NH_4F + 0.1N HCl at 1:10 ratio
[2] Soil shaken 15 minutes with 0.1N NHAc + 0.05N H_2SO_4 at 1:10 ratio

fertilizer distributor, attached to a manual-feed potato planter, was used to place the weighed fertilizer in bands 1 inch below and 2 inches on each side of the seed pieces.

The two centre rows of each plot, excluding 1½ feet at each end of each plot, were harvested with a tractor-mounted, one-row digger. Yield of tubers larger than one and three-quarter inches was calculated. A sample of 15-20 tubers was selected from each plot for cooking quality evaluation. The quality evaluations of the dry matter percentages and consumer preference indices were determined following a 40°F. storage period of not less than 6 weeks. Dry matter percentage was determined by the specific gravity method of Von Scheele *et al.* (16). The consumer preference index of the cooked tubers was determined by a panel of three people who rated each of the characteristics—texture, flavour and colour—on the basis of "40" for the most desirable and "10" for the least desirable. Prior to rating, the unpeeled tuber samples were boiled in "unsalted" tap-water until cooked. The skin was removed and a cooling period of 15 minutes was allowed before the cooked tubers were mashed with a fork.

TABLE 2.—AVERAGE YIELD AND EVALUATION OF THE COOKING QUALITY OF POTATOES BY (1) PER CENT DRY MATTER (BY THE SPECIFIC GRAVITY TEST) AND (2) CONSUMER PREFERENCE INDEX (BY THE COOKING TEST) FOR THREE SOILS AND THREE RATES OF APPLIED NITROGEN, PHOSPHORUS AND POTASSIUM CHLORIDE IN 1955

Soil type	Fertilizer applied (lb./acre)	Yield (bu./acre)	Evaluation of the cooking quality				
			Dry matter (%)	Cooking test			
				Texture (40)	Flavour (40)	Colour (40)	C.P. Index (120)
Fox sandy loam	N – 0	136	16.9	28.8	30.9	30.0	89.7
	N – 25	135	16.5*	27.8	30.4	30.2	88.4
	N – 50	150**	16.6	27.3	29.8	31.4	88.5
	P_2O_5 – 0	123	16.8	27.9	29.8	29.0	86.7
	P_2O_5 – 50	144**	16.4	27.2	30.7	31.6*	89.5*
	P_2O_5 – 100	154**	16.9	28.6	30.7	31.1*	90.4**
	K_2O – 0	134	17.3	29.0	29.8	28.9	87.7
	K_2O – 50	139	16.5**	27.4*	30.7	31.0*	89.1
	K_2O – 100	148**	16.2**	27.2*	30.7	31.8**	89.7*
Guelph sandy loam	N – 0	211	19.0	33.4	35.2	33.4	102.0
	N – 25	233*	19.2	33.6	35.9	33.4	102.9
	N – 50	242**	19.3	33.3	35.7	33.1	102.1
	P_2O_5 – 0	208	19.2	33.2	36.5	33.1	102.8
	P_2O_5 – 50	230*	18.9*	33.4	34.3*	32.7	100.4*
	P_2O_5 – 100	248**	19.5*	33.7	35.9	34.2	103.6
	K_2O – 0	201	19.5	33.9	36.1	33.3	103.3
	K_2O – 50	233**	19.1**	33.6	35.7	32.9	102.2
	K_2O – 100	252**	18.9**	32.9	35.0	33.8	101.7
Honeywood silt loam	N – 0	337	21.3	36.7	37.1	35.7	109.5
	N – 25	361*	21.2	36.7	37.2	34.7	108.6
	N – 50	398**	21.1	35.8	36.9	34.7	107.4*
	P_2O_5 – 0	356	21.3	36.8	37.1	35.1	109.0
	P_2O_5 – 50	361	21.0	35.6	37.1	35.2	107.9
	P_2O_5 – 100	379**	21.1	36.3	37.0	34.7	108.0
	K_2O – 0	357	21.4	36.3	37.1	34.8	108.2
	K_2O – 50	365	21.1*	36.0	36.9	34.9	107.8
	K_2O – 100	373	21.0**	36.2	37.2	35.3	108.7

* Significantly different from check at P = .05
** Significantly different from check at P = .01

RESULTS

The data in Tables 2 and 3 show that, on the Fox sandy loam (1955) and the Bookton loam, which produced little or no increase in yield due to nitrogen, the application of nitrogen fertilizer only decreased the dry matter percentage from 16.9 to 16.5 and 18.5 to 17.9 respectively. On the Bookton loam, application of nitrogen in excess of the amount required for maximum yield apparently adversely affected the dry matter content of the tubers. Moreover, on the Bookton soil, application of nitrogen fertilizer slightly reduced the consumer preference index. Increases in yield, due to nitrogen

TABLE 3.—AVERAGE YIELD AND EVALUATION OF THE COOKING QUALITY OF POTATOES BY (1) PER CENT DRY MATTER (BY THE SPECIFIC GRAVITY TEST) AND (2) CONSUMER PREFERENCE INDEX (BY THE COOKING TEST) FOR FOUR SOILS AND THREE RATES OF APPLIED NITROGEN, PHOSPHORUS AND POTASSIUM CHLORIDE IN 1956

Soil type	Fertilizer applied (lb./acre)	Yield (bu./acre)	Evaluation of the cooking quality				
			Dry matter (%)	Cooking test			
				Texture (40)	Flavour (40)	Colour (40)	C.P. Index (120)
Bookton loam	N – 0	154	18.5	34.7	33.9	35.5	104.1
	N – 25	162	18.4	35.0	33.4	35.5	103.9
	N – 50	170	17.9*	34.2	32.7	35.0	101.9*
	P_2O_5 – 0	108	18.0	34.2	33.0	34.4	101.6
	P_2O_5 – 50	181**	18.4*	35.0	33.7	35.7*	104.4**
	P_2O_5 – 100	197**	18.4*	34.8	33.3	36.0*	104.1*
	K_2O – 0	146	18.9	34.8	32.5	34.9	102.2
	K_2O – 50	166**	18.4*	34.8	33.3	35.3	103.4
	K_2O – 100	173**	17.5**	34.3	34.1*	35.9	104.3*
Tioga loam	N – 0	211	19.1	35.3	34.5	34.1	103.9
	N – 25	272**	19.6*	36.7*	36.4*	35.7*	108.8**
	N – 50	312**	19.3	35.7	34.8	36.0*	106.5**
	P_2O_5 – 0	218	19.1	35.3	35.8	35.5	106.5
	P_2O_5 – 50	285**	19.4	35.8	36.7	35.4	107.9
	P_2O_5 – 100	293**	19.5*	36.6*	36.1	34.8	107.5
	K_2O – 0	198	19.5	35.6	35.8	35.1	106.5
	K_2O – 50	283**	19.1*	36.0	36.1	34.9	107.0
	K_2O – 100	315**	19.3	36.1	36.7	35.8	107.6
Fox sandy loam	N – 0	229	19.1	34.0	33.2	35.7	102.9
	N – 25	254**	18.7	33.8	33.8	35.4	103.0
	N – 50	253**	18.8	33.1	33.4	35.5	102.0
	P_2O_5 – 0	214	18.1	33.2	33.2	35.7	102.1
	P_2O_5 – 50	257**	19.3*	32.6	33.2	35.4	101.2
	P_2O_5 – 100	264**	19.2*	35.1*	34.3	35.5	104.9*
	K_2O – 0	229	19.8	33.8	33.2	35.7	102.7
	K_2O – 50	239	18.7**	34.2	33.5	35.4	103.1
	K_2O – 100	258*	18.1**	32.9	34.0	35.6	102.5
Honeywood silt loam	N – 0	269	21.1	36.8	36.7	37.2	110.7
	N – 25	314**	20.9	36.4	36.9	37.2	110.3
	N – 50	331**	20.9	36.0	36.9	37.3	110.2
	P_2O_5 – 0	274	20.8	36.2	36.5	37.1	109.8
	P_2O_5 – 50	307*	20.9	36.3	37.0	37.3	110.6
	P_2O_5 – 100	330**	21.2*	36.7	37.0	37.3	111.0
	K_2O – 0	240	21.3	35.9	36.1	36.9	108.9
	K_2O – 50	317**	21.0	35.8	37.1	37.3	110.2*
	K_2O – 100	357**	20.5**	36.6	37.3	37.5	111.5*

* Significantly different from check at P = .05
** Significantly different from check at P = .01

fertilizer application, occurred on the Guelph sandy loam, Tioga loam, Fox sandy loam (1956) and the Honeywood silt loam (1955 and 1956). On these soils nitrogen applications had no effect on the dry matter percentage of the tubers, with the exception of the Tioga loam where an increase in dry matter percentage occurred with an application of 25 lb. of nitrogen. A slight decrease in consumer preference index occurred on the Honeywood silt loam (1955). However, the reverse was true on the Tioga loam where the application of nitrogen improved the texture, flavour and colour of the cooked tubers and resulted in an improvement in the consumer preference index.

Phosphorus fertilization increased yields on all soils in both 1955 and 1956, as shown in Tables 2 and 3, and also resulted in increases in dry matter percentage on all four soils in 1956. On the three soils in 1955, phosphorus had either no effect, or small, inconsistent effects on the dry matter percentage. There was an improvement in the consumer preference index due to phosphorus application on the Fox sandy loam (1955, 1956) and Bookton loam. On the other soils, changes in the consumer preference index due to phosphorus were generally not obtained except for the Guelph sandy loam in 1955 where there was a decrease in the index at the 50-lb. application rate.

On all soils potassium chloride fertilizer reduced the dry matter percentage of the tubers. However, this reduction was less on the Tioga loam location, rated low in the potassium soil test (Table 1). The decreases in the dry matter percentage of the tubers were lowest on those soils that produced the greatest yield increases due to potassium chloride. However, no correlation could be established between the degree of yield increase and the dry matter decrease, or between the initial soil test level of potassium and the decrease in the dry matter percentage of the tubers. On the other hand, there was an improvement in the consumer preference index due to application of potassium chloride fertilizer on the Fox sandy loam (1955), Honeywood silt loam (1956), and the Bookton loam. This is mainly accounted for by an improvement in the colour and/or flavour ratings with potassium chloride applications. On the other four soils, changes in the consumer preference index did not exist, even though there was a reduction in the dry matter percentage of the tubers on each.

DISCUSSION AND CONCLUSIONS

Evidence has been presented to show that consistent and appreciable increases in the yield of potatoes may be obtained from the use of relatively low amounts of the major plant nutrients on some of the potato soils in Ontario. In this study the changes in the per cent dry matter of the potato tubers, due to application of the fertilizer nutrients—nitrogen, phosphorus and potassium chloride—are in close agreement with those reported by Terman and co-workers (12, 13, 14). While the dry matter percentage data, shown in Tables 2 and 3, may indicate support for increased use of phosphorus and decreased use of nitrogen and potassium chloride fertilizers to improve the cooking quality of potatoes, it should be emphasized that the

reductions in the dry matter percentages are relatively small and should not generally be used as a basis for changing fertilization recommendations or procedures.

The studies of Kirkpatrick (10) and others (2, 8, 9) indicate that the cooking quality of potato tubers is a function of the characteristics—texture, flavour and colour. Using the consumer preference index method to evaluate the quality of the cooked product, the results obtained in this study, as they pertain to the fertilizer nutrient potassium chloride, differ markedly from those obtained by the per cent dry matter method. The cooking quality was either improved or unchanged due to potassium chloride fertilization. Where improvement in cooking quality occurred, it was associated with an improvement in colour and/or flavour. This is in agreement with the results obtained by Hill (7). The reduction in the per cent dry matter of potatoes due to potassium chloride, as reported by Terman (12), may not have been considered serious, in respect to their cooking quality, if this quality had been evaluated by a sensory method such as the consumer preference index. However, both methods are in close agreement in the evaluation of the effects of both nitrogen and phosphorus fertilizers on the potato cooking quality. For the rates and analyses of the fertilizer elements used in this study, the consumer preference index data suggest that good potato cooking quality is compatible with good yields.

Regardless of the method used in the evaluation of the potato cooking quality, the variations in quality associated with the fertilizer elements were relatively small within a given soil. However, among soils and between years, the variations in quality were much greater. Rainfall was abnormally low on the Fox sandy loam in 1955 but near normal in 1956. This was an important factor in the variation in the quality results between years. Harward *et al* (4) have shown that, when potatoes are grown under drought conditions, they are abnormally low in per cent dry matter. In this study it was noted that potatoes of a more uniform and better quality were produced on the Honeywood silt loam, where the rainfall was near normal for the two successive years.

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THE INHERITANCE OF RESISTANCE TO BACTERIAL WILT (*ERWINIA TRACHEIPHILA* (E.F.SM.) HOLLAND) IN CUCUMBER[1]

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ABSTRACT

The inheritance of resistance to bacterial wilt in cucumber was studied in the cross Marketer x P.I. 200818 by observing the reaction of P_1, F_1, F_2 and B_1 seedling populations in the greenhouse to inoculations with *Erwinia tracheiphila* (E.F.Sm.) Holland. The results show that resistance to bacterial wilt in the P.I. 200818 cucumber is due to a single dominant gene.

INTRODUCTION

Bacterial wilt, caused by *Erwinia tracheiphila* (E.F.Sm.) Holland, is a common disease of cucumbers and one of the most destructive of this crop. According to Walker (7) the disease occurs throughout the United States and Canada east of the Rocky Mountains and has been reported in several other countries. Lavallee *et al.* (5) report that in Canada, in 1945, up to 50 per cent infection and 30 per cent destruction of cucumbers by bacterial wilt occurred in certain locations. In some seasons, the disease has made cucumber growing unprofitable in localized areas.

The disease is transmitted (7) by the striped cucumber beetle (*Acalymma vittata* Fabricius) and the spotted cucumber beetle (*A. duodecimpunctata* Olivier). Infected plants wilt as a direct result of insufficient water movement, caused by mechanical plugging of the xylem by bacteria (3). Plants may be destroyed by the disease at any time in their development from the early seedling to the late fruiting stage. Observations made at the beginning of this study indicate the possible importance of the effect of light, temperature and other factors upon the development of the disease. Wei *et al.* (8) discuss the relationship of host nutrition to the rate of development and severity of bacterial wilt in *Cucurbits*.

At present, the only recommended method of control of the disease is by eradication of the cucumber beetles. This is a costly item in cucumber production in areas where the incidence of the beetles is normally high, since insecticides must be applied early and frequently for effective control. While Thompson (6) and Walker (7) mention that the inclusion of a fungicide with the insecticide affords a measure of disease control, and more recent research (9) indicates that field test sprays of either streptomycin or terramycin reduced the incidence of the disease resulting in yield increases of up 25 per cent, no definite recommendations are yet offered along these lines. It is apparent, therefore, that the development and use of resistant varieties would be the most satisfactory method of controlling bacterial wilt in cucumber.

[1]Contribution No. 928 from the Horticulture Division, Experimental Farms Service, Canada Department of Agriculture, Ottawa, Ont.

Resistance to bacterial wilt in cucumber was reported as early as 1939 (1) when an F_1 hybrid, Tokyo Long Green x Vickery Forcing, was observed to be resistant to *B. tracheiphila* E.F.Sm. Walker (7) stated that there are varietal differences in susceptibility to the disease and indicated that varieties may be improved in resistance by breeding. In 1953, Ferguson, Lyall and Jasmin (2) observed varying degrees of resistance to bacterial wilt in a screening test of more than 1,000 plants of *Cucumis* species.

In 1956, Jasmin and Lyall (4) found a high degree of resistance to bacterial wilt in United States Department of Agriculture Plant Introduction 200818 (originating in Burma) which was among several species of cucumber included in a further greenhouse screening test at Ottawa. Wilson *et al.* (10) in 1956 also found P.I. 200818 to be resistant to bacterial wilt.

P.I. 200818 cucumber was used as the resistant parent in this study of the inheritance of resistance to bacterial wilt which was made to facilitate the development of resistant varieties.

MATERIALS AND METHODS

To determine whether the resistance to bacterial wilt in the P.I. 200818 cucumber was dominant or recessive, the varieties Marketer, Ottawa 57G-205-3, Wisconsin SMR 12 and the F_1 progenies of each of these parents crossed with the resistant parent were observed in the greenhouse for their reaction to inoculation with bacterial wilt. The results of this test are shown in Table 1.

On the basis of its wide usage as a cucumber for the market and home garden, Marketer was selected as the susceptible variety in this study. F_2 seedling progenies of the cross Marketer x P.I. 200818 and the first backcross progeny (B_1) of (Marketer x P.I. 200818) F_1 x Marketer were studied for their reaction to inoculation with bacterial wilt, also in the greenhouse. Three separate tests of F_2 populations were made while a limited amount of seed permitted only a single test of the B_1 population. Inoculated and uninoculated control samples of Marketer were included in each test.

TABLE 1.—REACTION OF CUCUMBER VARIETY AND F_1 HYBRID SEEDLINGS TO ARTIFICIAL INOCULATION WITH BACTERIAL WILT IN THE GREENHOUSE

Variety or hybrid	Number of Plants		
	Inoculated	Resistant	Susceptible
P.I. 200818	78	78	0
Marketer	72	0	72
Ott. 57G-205-3	23	0	23
Wisc. SMR 12	15	0	15
Marketer x P.I. 200818 F_1	27	27	0
Ott. 57G-205-3 × P.I. 200818 F_1	24	24	0
Wisc. SMR 12 × P.I. 200818 F_1	26	26	0

Small lots of seed of the parent varieties and the F_1 progenies were sown in 8-inch bulb pans with the stand averaging 15 to 25 seedlings per pan, while the F_2 and B_1 populations were grown in 12 x 24-inch flats with an average of 40 to 50 seedlings per flat.

The inoculum was supplied by the Division of Botany and Plant Pathology, Science Service, Canada Department of Agriculture, Ottawa, and was prepared from a culture of *Erwinia tracheiphila* (E.F.Sm.) Holland, isolated from diseased cucumbers grown in the Ottawa area in 1953. Inoculation was done at the first true-leaf stage of development with a B-D Yale syringe using a 1-inch hypodermic needle. The needle was inserted under the upper epidermis of a cotyledon and a few drops of the bacterial suspension were introduced into the seedling. All inoculations were carried out by one operator to minimize error.

The temperature in the greenhouse during the testing periods ranged from 75 to 90 degrees Fahrenheit. Since these tests were carried on during a period of short days, supplemental incandescent lighting was supplied to provide daily light periods of 14 hours.

Following the date in each test on which the first susceptible seedlings succumbed to bacterial wilt, plant counts were made at 4- to 6-day intervals, depending upon the rate of development and severity of the disease. All susceptible plants which were entirely wilted and beginning to desiccate were removed from the containers at each inspection. The seedlings were classified as either resistant or susceptible with no gradations.

RESULTS AND DISCUSSION

During periods of bright sunny weather symptoms of bacterial wilt appeared 10 to 12 days following inoculation, while the development of the disease was delayed by as much as an additional 2 weeks when the weather was consistently dull and cool. No seedling recovered from the disease following the appearance of the initial symptoms but the disease developed in varying periods of time until the plant wilted completely and

TABLE 2.—SEEDLING REACTION OF F_2 PROGENY OF MARKETER X P.I. 200818 AND THE B_1 PROGENY OF (MARKETER X P.I. 200818) F_1 X MARKETER TO ARTIFICIAL INOCULATION WITH BACTERIAL WILT IN THE GREENHOUSE

Generation	Test series	Number of Plants		X^2	P
		Resistant	Susceptible		
F_2	I	536	161	1.343	.20–.30
F_2	II	110	33	0.280	.50–.70
F_2	III	89	27	0.183	.50–.70
B_1	I	42	47	0.280	.50–.70

died. The results of the test of the varieties and F_1 hybrids presented in Table 1 indicate that with complete destruction of the susceptible varieties conditions in the greenhouse were favourable for the development of the disease.

Table 1 shows that P.I. 200818 was highly resistant to bacterial wilt and that this resistance was transmitted to the F_1 progenies of susceptible varieties crossed with P.I. 200818. These results also show that, under the conditions of this test, resistance to bacterial wilt was dominant to susceptibility.

Table 2 shows that the observed distribution for resistant and susceptible segregates for each test of the F_2 populations was similar to that expected on the basis of a 3:1 ratio. In the single test of the B_1 progeny the segregation is in satisfactory agreement with a 1:1 ratio as is indicated by the X^2 and P values. Therefore it can be concluded that resistance to bacterial wilt in P.I. 200818 when in combination with the variety Marketer is controlled by a single dominant gene.

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EVALUATION OF ALFALFA FOR RESISTANCE TO BACTERIAL WILT IN FIELD AND GREENHOUSE TESTS[1]

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ABSTRACT

This paper describes the application of improved methods to large scale field and greenhouse tests of alfalfa for resistance to bacterial wilt, *Corynebacterium insidiosum* (McCull.) Jenson. In field tests rooted cuttings or seedlings were inoculated by the bare-root soak method when planted in the field in May and by hypodermic injection of each root in the fall. In the following spring or fall the plants were cut off below ground with a special blade, pulled and individually evaluated for wilt resistance. In the greenhouse the root-ball soak method of inoculation was used and readings of seedlings were made after 3 months.

Greenhouse tests were as reliable as those obtained in the field, and were particularly useful for rapid screening of large populations. Field tests proved desirable for simultaneous studies on wilt resistance, growth habit, winter hardiness, and other qualities, and for final evaluation of potential variety material.

INTRODUCTION

When bacterial wilt became a major limiting factor in the production of irrigated alfalfa in Alberta (6), a program for testing and development for resistance to this disease was started at Lethbridge as part of the co-operative work on the improvement of alfalfa in Canada (2). In this program populations of breeding stock from western Canada and the United States were screened for wilt resistance.

Studies on methods and materials for testing alfalfa for resistance to bacterial wilt have been recently reported (3). This paper describes the application of these methods on different types of material and compares results from large scale greenhouse and field experiments.

FIELD TESTS

The field tests were initiated in 1945 in a wilt elimination nursery at the Experimental Farm, Lethridge, Alberta. This nursery consisted of about 2 acres of land on which severe damage from bacterial wilt had occurred previously. Water was readily available from an independent irrigation ditch. The soil was a heavy silt loam with a clay subsoil of high moisture retention capacity.

Material Tested in Wilt Elimination Nursery

The alfalfa evaluated for resistance to bacterial wilt consisted of local wilt-free selections from old variety test plots and breeding material introduced from experimental farms and universities in western Canada

[1] Joint contribution from the Forage Crops Division, Experimental Farms Service, and Botany and Plant Pathology Division, Science Service, (Contribution No. 1643), Canada Department of Agriculture, Ottawa.

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FIGURE 1. First inoculation was made by bare-root soak method immediately before planting in wilt elimination nursery.

and the United States. Most of the local material was obtained from 5-year-old variety test plots at Lethbridge and an observation nursery at Brooks, Alberta. Plants free from bacterial wilt and crown diseases were selected for propagation. Crown cuttings from each selected plant were

FIGURE 2. General view of wilt elimination nursery.

FIGURE 3. The second inoculation was made in the fall by hypodermic injection of each root.

rooted in moist sand in the greenhouse during the winter. When necessary, additional cuttings were later made from the stems of the new growth to ensure that 10 clones of each plant would be available for test.

Methods of Inoculating and Handling of Plants

All plants tested for wilt resistance in the field were grown from cuttings or seed in the greenhouse during the winter and were at least 3 months old when the tests were started in the spring. They were inoculated by the bare-root soak method (3) immediately before planting in the field (Figure 1). The inoculated plants were wrapped in bundles covered by moist paper towelling for transport to the wilt nursery and were planted 1 foot apart in rows 3 feet apart. At least 10 cuttings from each clonal selection and 20 seedlings from each seedling line were planted successively in rows (Figure 2). Preliminary tests had indicated that conditions for infection were uniform over the field and replication other than within the plot was not necessary.

The tests were usually started in early May, or as soon as irrigation water was available. Five irrigations were made during the first season. In early years the water was applied by flooding but later by sprinkling. Disease development was also encourged by the clipping of the plants at about monthly intervals.

A second inoculation to eliminate escapes was made in the fall by hypodermic injection of each root (Figure 3) (3). The plants were rated in the field for quality, forage production, and spring recovery.

Lifting and Evaluation of Plants

The plants were lifted for final rating in the spring or fall of the second year. If maximum disease development in tolerant material was desired

FIGURE 4. By the second season only resistant material survived in the wilt elimination nursery.

(Figure 4) the tests were continued for two full growing seasons. To lift the plants, a U-shaped blade, 1 foot wide and 2 feet deep was mounted on a small tractor (Figure 5) and pulled down the rows so that the roots were cut off about 6 inches below the surface (Figure 6). The plants were then rated individually for wilt infection as previously described (Figure 7) (3). Clonal lines with any trace of infection were discarded. Wilt-free

TABLE 1.—NUMBERS OF WILT-FREE PLANTS SELECTED FROM EIGHT ALFALFA VARIETIES FOR RETESTING IN A FIELD NURSERY, AND PERCENTAGE RETAINED AS WILT FREE, 1946-50

Variety	Number of selections tested in nursery						
	1946	1947	1948	1949	1950	Total	Per cent retained
Cossack			1		19	20	55
Hardistan				14	18	32	59
Ladak	40	35	36	10	14	135	34
Orestan				7		7	29
Ranger	17	2	12	31	18	80	54
Rhizoma			6	1		7	0
Viking	17	36	22	5	20	100	52
Wisconsin selection			13	62		75	36
Totals	74	73	90	130	89	456	44

FIGURE 5. Tractor-mounted blade for cutting roots 6 inches below surface.

FIGURE 6. Plants and field markers left in original location while roots are cut.

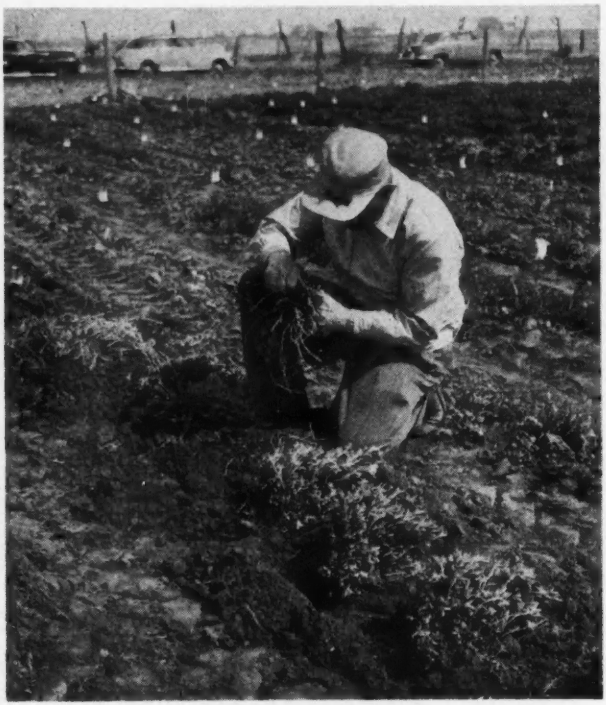

FIGURE 7. Root-cut plants are pulled and rated for wilt infection.

lines were saved and those with sufficiently high evaluation for forage yield, quality, and other characteristics were included in the polycross nursery. In seedling lines, an allowance was made for a variable but low degree of infection whereby wilt-free plants from progenies with relatively little infection were retained for further testing. Alfalfa varieties or stains were evaluated for wilt resistance by determining the average infection ratings and percentages of infected plants.

Results

During the first 5 years of the program (1946-1959), approximately 50,000 alfalfa plants were tested in the wilt elimination nursery. About two-thirds of these plants were screened for the breeding program and the remainder were varieties and strains. Since 1950 more of the preliminary screening has been done in the greenhouse and less in the field. There were 456 single-plant selections from old variety test plots representing eight varieties and strains (Table 1). Over one-half of the selections taken from Cossack, Hardistan, Ranger, and Viking remained wilt-free. All selections from the highly susceptible variety Rhizoma proved to be escapes.

GREENHOUSE TESTS

The field tests required 12 to 18 months to complete. This caused a great delay in the assessment of breeding material, especially if large populations of seedlings had to be screened. Consequently, greenhouse tests for wilt resistance were investigated in an attempt to speed up the program.

Methods and Materials

The methods of inoculation used in the greenhouse tests have been previously reported (3). In most tests 20 seedlings were grown or 10 rooted cuttings were planted in 6-inch pots of 3:1 soil-sand mixture with five replicates. After about 2 months of growth the plants were inoculated by the root-ball soak method with inoculum prepared from diseased plants. To encourage disease development the plants were kept in a warm greenhouse, heavily watered and cut back about once a month. Three months after inoculation all plants were removed and evaluated for infection and damage. With varieties and strains, each plant was rated for wilt infection by means of a 0 to 5 scale. When large populations of plants were screened, however, only the wilt-free, slight, and severe categories were used.

A wide range of alfalfa material from various sources was successfully tested for wilt resistance in the greenhouse, including several strains of *Medicago falcata*. Large populations of progenies from crosses and plants in polycross nurseries were screened for resistance.

Results

Varieties of alfalfa tested in the greenhouse showed the same general reaction to bacterial wilt as those grown in the field (6), even when the readings were taken 8 weeks after inoculation (Table 2). In this representative test the infection ratings and percentages of infected plants indicated that Ranger and Buffalo were highly resistant, Hardistan, Ladak, Cossack, and Viking were moderately resistant, and Grimm, Rhizoma, and a strain of *M. falcata* were highly susceptible.

TABLE 2.—INITIAL REACTION OF ALFALFA VARIETIES TO BACTERIAL WILT IN GREENHOUSE TEST AT LETHBRIDGE (8 weeks after inoculation)

Variety	Percentage of infected plants[1]	Average infection rating[1]
Ranger	20.2	0.3
Buffalo	29.4	0.5
Hardistan	38.7	0.6
Ladak	40.9	0.6
Viking	41.3	0.8
Cossack	57.9	0.8
Rhizoma	71.0	1.2
M. falcata (S. 3009)	60.6	1.4
Grimm	74.8	1.6

[1] Least significant differences (1% level): Percentage of infected plants—19.2 per cent. Average infection rating—0.3.

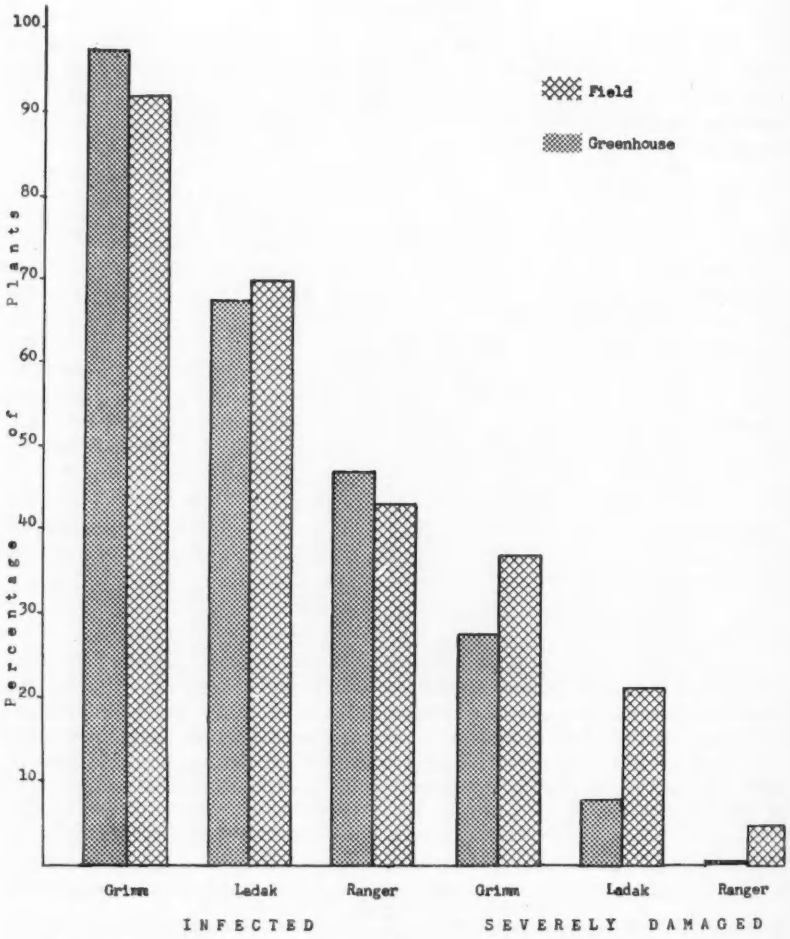

FIGURE 8. Reaction in greenhouse and field of three alfalfa varieties to bacterial wilt.

As shown in Figure 8, the degree of infection 3 months after inoculation in the greenhouse was approximately equal to that obtained in a year after inoculation in the field. In this series of tests with Grimm, Ladak, and Ranger, however, more plants were severely damaged in the prolonged field tests than in the greenhouse. The relative resistance of these varieties was similar in the greenhouse and field.

Few escapes occurred in greenhouse screening tests on large populations of plants. When 325 progenies from plants in a polycross nursery were tested, 200 lines proved highly resistant to bacterial wilt. All plants in

these lines that were wilt-free or slightly infected were re-inoculated and none became severely infected. In another test only 1 per cent of the plants that remained wilt-free in the greenhouse became infected after they were re-inoculated and grown in the field for a year.

DISCUSSION

This study provided an opportunity to compare the value of field and greenhouse methods of testing alfalfa for resistance to bacterial wilt. In general, the results indicate that both kinds of test are reliable and useful and that one may sometimes supplement the other. Factors that can determine the choice of test include the object of the testing program, type of material, conditions desired, space and facilities available and the urgency with which the results are required.

Field tests for wilt resistance have been widely used in the United States (1, 4, 5) and have aided greatly in the development of several resistant varieties of alfalfa. They also have proved of great value at Lethbridge, especially for verifying the resistance of wilt-free plants selected in old variety plots. They have thus speeded up the process of natural selection in an aging stand, a process studied previously by Battle (1). Advantages of the field tests over greenhouse tests as revealed at Lethbridge are the higher degree of damage caused to susceptible plants and the opportunity in the field for simultaneous rating of wilt resistance, growth habit, winter hardiness, and other qualities. Also, the final evaluation of potential new varieties should be made under field conditions. The major disadvantages of the field tests are the necessity of two inoculations to eliminate escapes and the time of at least one year required for final evaluation of the material. This long period has caused considerable delay in the breeding program. The longer time required for completion of field tests at Lethbridge as compared to Nebraska and other areas may be at least partly explained by the relatively low soil temperature during the growing season at Lethbridge.

The greenhouse tests had the great advantage of requiring only about one-quarter of the time necessary for a field test at Lethbridge. The root-ball soak method of inoculation can be easily applied and allows few escapes. In the greenhouse large quantities of material can be screened rapidly for wilt resistance at any time of the year when space is available. Also, the temperature, soil moisture, and other factors can be controlled much more closely in the greenhouse than in the field. The greenhouse method is particularly suitable for rapid preliminary screening of large populations of seedlings and it can thus greatly accelerate the breeding program for resistance in alfalfa.

ACKNOWLEDGEMENT

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GENETIC ANALYSIS OF WHEAT CHROMOSOMES

I. DESCRIPTION OF PROPOSED METHODS[1]

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ABSTRACT

The F_1 obtained from crossing a substitution line with the recipient variety will be heterozygous only for genes on one pair of chromosomes. Crossing-over at meiosis, therefore, will result in recombinations only with respect to genes carried by this pair of chromosomes. The products of such recombinations will be obtained as univalent chromosomes of monosomics when such F_1's are used to pollinate a line of the recipient variety deficient (nullisomic or monosomic) for the chromosome under study. When the monosomics so obtained are individually selfed, approximately 25 per cent of the offspring will be disomic, and homozygous for the genetic constitution of the backcross monosomics. The relative frequencies of progenies with different genetic constitutions will indicate directly the number and arrangement of genes differentiating the donor from the recipient variety chromosome.

Genetic differences can be determined specifically for chromosome arms if, instead of whole-chromosome deficient lines of the recipient variety, those with telocentrics are used.

The possible usefulness of the method for breeding, as well as studies of evolution, is briefly discussed.

INTRODUCTION

The whole-chromosome deficient series developed by Sears (6, 7) in the variety Chinese of common wheat, and related derived lines, make possible more precise genetic studies in this organism than are possible by conventional methods. The nullisomics themselves, through phenotypic modifications, revealed the chromosomes responsible for genetic control of some characters (7, 8). In a number of studies (1, 5, 8, 11), F_2 and F_3 populations of crosses involving the Chinese lines with varieties having contrasting characters were used to associate a number of characters with specific chromosomes. Modifications of the normal ratios caused by the characteristic transmission behaviour of the univalent chromosomes, revealed the critical chromosome(s) in these studies. Substitution lines, that is lines in which the chromosomes of a donor variety are separately tested against the genetic background of the recipient variety, have permitted the unambiguous identification in a number of varieties of the chromosomes that carry genes for stem rust resistance (4, 8, 9, 10). Such lines also clearly revealed that four chromosomes were responsible for the difference in leaf rust reaction of the varieties Chinese and Thatcher (12). The striking genetic effects of substituted chromosomes on such characters as yield, earliness, height, lodging resistance, etc., obtained by Kuspira and Unrau (2), indicate the value of substitution lines in studying the genetics of quantitative characters. Kuspira and Unrau (3) have shown how substitution lines, when crossed with the recipient variety, will show

[1] Contribution from the Department of Plant Science, University of Alberta, Edmonton, Alta. Investigations with wheat aneuploids are supported by an Extra-mural Research Grant from the Cereal Division, Canada Department of Agriculture, and a Grant-in-aid of Research from the National Research Council of Canada.

[2] Professor of Plant Science.

in the F_1, F_2 and F_3 the number and dominance relationships of genes on a substituted chromosome.

In the following, a brief description will be given of methods by which the number and recombination relationships of genetic effects of individual common wheat chromosomes may be determined. That is, the methods outlined should make possible a systematic and complete mapping of the chromosomes of common wheat. Examples and symbols have been arbitrarily chosen to illustrate the procedures rather than to represent actual situations. While no actual "linkage maps" are presented, the methods outlined and the nature of results anticipated are not based on speculation. All procedures, in principle, are at present used for other purposes by numerous investigators and the findings anticipated are based on the well-known characteristic breeding behaviour of univalent chromosomes in wheat monosomics.

MATERIALS AND PROCEDURES

To illustrate the principle it will be assumed that significant genetic effects are known to be associated with a certain chromosome from studies of substitution lines. The donor variety from which the chromosome was transferred will be referred to as "D"; the recipient variety as "R"; the chromosome concerned as "I" and the genotypes will be "D(IABC)", representing genes on chromosome "I" of the substitution line and "R(Iabc)" representing the recipient variety.

A. *Tests Involving Whole Chromosomes*

Step 1. The substitution line is crossed with the recipient variety. F_1 produced will be heterozygous only for genes on chromosome I and can be illustrated as F_1 $\frac{D(IABC)}{R(Iabc)}$.

On this pair of chromosomes only, crossing over will result in recombinations. With three loci eight combinations are possible. Obviously, 2^n would give the possible combinations with more loci (n = number of loci).

Step 2. The F_1, used as the male, is crossed with nullisomics or monosomics of I of the recipient variety. In each of the monosomic backcross progeny the univalent chromosome will be the product of crossing over and recombination of the F_1. There will, obviously, be as many genetically distinct and different univalents as there are recombinations. With three loci involved there would be eight genetically different univalents.

Step 3. The monosomic backcross plants are kept separate and permitted to self. The disomic progeny obtained from this selfing (approximately 25 per cent) will be completely homozygous. As many as necessary of these families, each tracing back to a different backcross monosomic, are increased and the required studies made. Mapping can then be done directly from the relative frequency with which different recombination classes are obtained.

B. *Tests Involving Chromosome Arms*

Telocentrics of one or both arms are now available for all chromosomes in the variety Chinese and it is consequently possible to determine which arm carries certain genes.

Step 1. The substitution line D(IABC) is crossed with a line of the recipient variety homozygous for a telocentric of chromosome I, and designated here as R(tIab_). The F_1 will be heterozygous for genes on the telocentric arm, and hemizygous for the genes of the donor variety located on the arm missing in the recipient line. The F_1 may be designated $\frac{D(IABC)}{R(tIab\_)}$. Crossing over and recombination can occur only between the telocentric and the homologous arm of the donor chromosome.

Step 2. The F_1, used as the male, is crossed to nullisomic or monosomic I of the recipient variety. There will be two types of monosomic backcross progeny: those with an entire univalent and those with a telocentric univalent. In the progeny having entire univalents, there will be as many genetic recombinations as there are heterozygous loci on the telocentric being tested. For the example used there can be the following four genetically different univalents: IABC, IAbC, IaBC and IabC. The arm for which the recipient line used in Step 1, if deficient, will in all cases be genetically identical with the homologous arm of the donor chromosome.

Step 3. Monosomic backcross progeny with entire univalents are permitted to self and the offspring from each are kept separate. All disomic progeny obtained will be identical with respect to genes on one arm. With respect to genes carried by the telocentric, there will be as many classes as there are recombinations, and the relative frequency of each class will indicate directly crossover relationships on the telocentric being tested.

With the information obtained from A, it should now be possible to indicate recombination relationships on both arms, if more than one locus was involved in both.

C. *Tests of Homologous Chromosomes from Different Donor Varieties*

Such studies may be necessary or desirable in a number of situations. Thus, similar quantitative genetic effects may be produced in the recipient variety by homologous chromosomes from different donor varieties and the objective might be to determine whether the same or different loci are involved.

Step 1. The two substitution lines are crossed.

Step 2. The F_1 is crossed to the appropriate nullisomic or monosomic of the recipient variety.

Step 3. Again the monosomic backcross progeny are permitted to self, and the families kept separate. The disomics in each are increased and appropriate comparative tests are made. If all the progeny are similar it may be assumed that the two homologous chromosomes from different donor varieties are genetically alike. If there are distinct recombination classes, it follows that on the two homologous chromosomes from different donor varieties there are different loci that produce the same effect.

DISCUSSION

Largely because of the great amount of genetic duplication chromosome mapping, except for a few simply inherited characters, is not feasible in common wheat by conventional genetic methods. Aneuploid lines and related types make possible the association of genes with specific chromosomes without the use of linkage testers. The methods here described should make possible the detailed mapping of the chromosomes of common wheat for qualitative and quantitative characters. Such information, when systematically assembled, will be of inestimable value to the wheat breeder. He would know the gene content of the chromosomes of distinctive varieties with respect to disease and insect resistance, quality, yield, etc. Moreover, it should be possible to establish the association of desirable with undesirable characters and the ease with which such association can be broken.

The principles of this method applied to a breeding program remove all of the disadvantages visualized by Kuspira and Unrau (2) for the whole-chromosome substitution method of breeding. Compared with the backcross method, the transference of genes from donor to recipient

variety by whole chromosome substitution is generally quicker since it is immaterial whether the genes concerned are dominant or recessive and consequently no inter-backcross selfings are necessary. Also, once desirable genetic effects are associated with a chromosome in a donor variety, no artificial nurseries or tests are needed while such a chromosome is being transferred to the recipient variety.

It is important to stress that linkages of desirable with undesirable genes can be broken (if breakable) and the products of such breakage can be more readily detected than by any other method. As outlined in A III, the possible recombination products involving the donor with the recipient chromosome are all homozygous. They are easily increased and effectively studied and those with the desirable recombinations can be selected.

Chromosome substitutions and the analysis of gene content of whole chromosomes should make possible a precise study of the evolutionary changes that have occurred in the chromosomes of common wheat. The chromosomes of various synthetic hexaploid wheats can be singly substituted for their homologues in cultivated wheat. The procedures here outlined will then enable a precise determination of the extent to which genetically the chromosomes of the ancestral and the cultivated forms differ. It will clearly reveal the basis of major as well as minor differences, and should permit the ready re-incorporation into common wheat of valuable genes from the ancestral form that may have been lost or changed during the period when wheat was cultivated by man.

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A RAPID METHOD FOR ESTIMATING THE OIL CONTENT OF SUNFLOWER SEEDS[1]

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ABSTRACT

A rapid method was developed for estimating the oil content of sunflower seeds. The method consisted of placing a given number of seeds between layers of filter paper, pressing them by means of a laboratory hydraulic press, and measuring the weight of oil absorbed by the filter paper. The effects of pressure-load, time of pressing, types of absorbing paper, size of sample, and dehulling of the seed were studied and a recommended procedure adopted. The oil values obtained by the rapid method were approximately 73 per cent as high as those obtained by the standard Soxhlet procedure. Analyses of 117 sunflower seed samples by the two methods gave a correlation coefficient of +0.852. The method is particularly suited to assessing of plant breeding selections for oil content because of its speed and its adaptability to small samples.

INTRODUCTION

Since 1946, sunflowers have been an important commercial crop in southern Manitoba. The main outlet for the crop has been the processing of the seeds for edible oil. Parallel to the commercial production, an extensive sunflower breeding program has been in progress at the Experimental Farm, Morden, Manitoba, for the purpose of developing improved varieties. One of the criteria of selection used in the breeding program is the oil content of the seed. Since several hundred selections are made each year, the task of measuring the oil content of each has been too time-consuming to provide results soon enough for planning the breeding program effectively for the next season. Further difficulties were encountered in the grinding of the seed samples because of the care required to obtain a ground sample truly representative of the combined hull and kernel.

Stefansson[3] applied a known pressure to a given weight of seeds folded in mimeograph paper. He found the loss in seed weight resulting from the oil absorbed by the paper was a promising indication of the seed oil content. In consultation with the authors, he suggested that further investigational work should be performed on the principle. The present study was conducted to evaluate the factors affecting the method and to establish a recommended procedure.

MATERIALS AND METHODS

A Carver Laboratory Press[4], with a piston 9 cm. in diameter, was used throughout. The sunflower seed sample was weighed to the nearest milligram, placed between layers of filter paper, then pressed to allow the paper to absorb the free oil. The seeds were again weighed and the loss

[1] Joint contribution from Horticulture Division (Contribution No. 942) and Forage Crops Division, Experimental Farms Service, Canada Department of Agriculture, Ottawa, Ont.

[2] Food Technologist and Agrostologist, respectively, Experimental Farm, Morden, Man.

[3] Stefansson, B. R., Department of Plant Science, University of Manitoba, Winnipeg, Man. *Personal communication.* 1953.

[4] Fred S. Carver, Inc., Summit, N.J.

in weight reported as per cent oil. Four separate experiments were conducted. All samples were in equilibrium with room atmosphere for several weeks and had uniform moisture content of 5.0 ± 0.5 per cent.

Experiment No. 1

This was a factorial experiment to determine the influence of certain factors on the proposed principle. A bulk sample of advance hybrid seed was used. All lots were pressed for 3 minutes. The treatments included:

(1) *Two pressure loads*—(a) 6,000 and (b) 10,000 lb.
(2) *Two types of paper*—(a) No. 1 mimeograph paper with three thicknesses on each side of the sample, and (b) Whatman No. 1 filter paper circles 9 cm. in diameter with two circles on each side of the sample.
(3) *Two conditions of seed*—(a) whole and (b) dehulled. Care was exercised in dehulling to ensure that the meats were neither fractured nor damaged.
(4) *Two sizes of sample*—(a) 10 seeds and (b) 25 seeds.

Triplicate pressings were performed for each combination of the above factors.

Experiment No. 2

Triplicate pressings were made on whole and on dehulled seeds of six different sample sizes. The sample sizes were 5, 10, 15, 20, 25 and 30 seeds. The pressure was 10,000 lb., applied for 3 minutes. Two circles of Whatman No. 1 filter paper on each side of the sample were used throughout. Seed from the same source as that in Experiment No. 1 was employed.

Experiment No. 3

This was a study of the effect of pressing time on the amount of oil absorbed by the paper. Again, whole and dehulled seeds were used. The seed source was the same as for Experiments No. 1 and No. 2. The conditions for this study were a 20-seed sample pressed between filter paper circles at 10,000 lb. load for 1, 2, 3, 4 and 5 minutes.

Experiment No. 4

From results of Experiment No. 1, 2, and 3, a tentative rapid procedure was adopted. This method was applied to a random group of 117 sunflower selections. The same samples were submitted to the Chemistry Division, Canada Department of Agriculture, Ottawa, Ontario, for analysis by the standard Soxhlet procedure (1). The results obtained by the two methods were correlated.

In addition, 27 selections, representing a wide range of seed sizes and also a wide range of seed oil content, were analysed at Morden in duplicate by both procedures. The 27 selections were made up of three different seed sizes and three levels of oil content. The seed sizes were as follows: *small*—30 to 50 grams per 1000 seeds; *medium*—50 to 70 grams per 1000 seeds; and *large*—70 to 95 grams per 1000 seeds. The levels of oil content by Soxhlet extraction were: *low*—14 to 19 per cent; *medium*—20 to 27 per cent; *high*—28 to 40 per cent

The mean values for each selection by the two methods were correlated. Fractional ratios of the oil values obtained by the press method and those by the Soxhlet method were compared for the three levels of seed size and of oil content.

TABLE 1—EFFECTS OF PRESSURE LOAD, TYPE OF PAPER, SEED CONDITION AND SEED NUMBER ON THE OIL PRESSED FROM THE SEEDS

Factor	Treatment	Mean oil value (%)	F.
Pressure load	6,000 lb.	20.08	8.05**
	10,000 lb.	21.76	
Type of paper	Filter	21.54	4.46*
	Mimeograph	20.30	
Seed condition	Whole	17.75	85.39**
	Dehulled	24.05	
Seed number	10 seeds	23.04	54.02**
	25 seeds	18.80	

* Significant at the 5% level
** Significant at the 1% level

TABLE 2.—EFFECT OF SAMPLE SIZE ON THE OIL PRESSED FROM WHOLE AND DEHULLED SEEDS

Sample size (no. of seeds)	Oil value (%)	
	Whole seeds	Dehulled seeds
5	24.73	27.47
10	21.80	25.77
15	20.63	24.80
20	19.00	23.77
25	18.37	23.63
30	16.70	22.40
F	8.49**	3.76*
L.S.D. (P .05)	3.01	2.86

* Significant at the 5% level
** Significant at the 1% level

EXPERIMENTAL RESULTS AND DISCUSSION

Experiment No. 1—Preliminary Factorial Study

A summary of the results from Experiment No. 1 is given in Table 1. All four factors had an effect on the amount of oil absorbed by the paper. The 10-seed sample gave a higher oil absorption than the 25-seed sample, presumably because of the higher pressure load applied per seed. Oil values, based on the original whole seed weight, were substantially higher for the dehulled seed than for the whole seed. Evidently, the hull reduced the absorption of oil by the paper. The higher pressure load of 10,000 lb. resulted in a higher oil value, although the mean difference between the two loads barely exceeded the 1 per cent level of significance. The difference between the two types of paper was significant at the 5 per cent level with a slightly higher value obtained for the filter paper. The interactions between factors were not significant. Variations caused by them were included in the experimental error.

TABLE 3.—EFFECT OF PRESSING TIME ON THE OIL REMOVED FROM WHOLE AND DEHULLED SEEDS

Pressing time (min.)	Oil value (%)	
	Whole seeds	Dehulled seeds
1	17:43	23.23
2	18.00	23.67
3	18.23	24.07
4	18.97	23.17
5	18.27	24.00
F	0.87[1]	0.36[1]

[1] Not significant

TABLE 4.—EFFECT OF SEED SIZE AND SEED OIL CONTENT ON THE RATIO OF THE OIL VALUE BY THE PRESS METHOD AND THAT BY THE SOXHLET METHOD

Seed size	Mean ratio	Oil content	Mean ratio
Small	0.805	Low	0.697
Medium	0.703	Medium	0.764
Large	0.682	High	0.729
F	15.74**		5.27*
L.S.D. (P .05)	0.046		0.046

* Significant at the 5% level
** Significant at the 1% level

Experiment No. 2—Effect of Sample Size

Variation in sample size (Table 2) showed that, with both whole and dehulled seeds, the oil values decreased as the number of seeds per sample increased. A sample of 15 to 20 seeds appeared to be optimal with respect to combining reliability with high oil value.

Experiment No. 3—Effect of Pressing Time

Varying the pressing time from 1 to 5 minutes gave no significant difference in the oil values from either whole or dehulled seeds (Table 3). A pressing time of 2 minutes was considered a safe choice for this factor.

While the oil values were substantially higher for dehulled seeds than whole seeds in Experiment No 1, 2, and 3, there was not sufficient improvement in precision to warrant the tedious practice of dehulling the seeds.

Experiment No. 4—Comparison of Adopted Press Method and Soxhlet Procedure

From results of Experiment No. 1, 2, and 3, the procedure that was arbitrarily accepted was a 20-seed sample pressed between filter paper at 10,000 lb. load for 2 minutes. The correlation coefficient calculated from the oil values obtained for the 117 selections by the press method and by the standard Soxhlet procedure was +0.852.

The correlation coefficient for the 27 selected samples analysed by the two methods was +0.937. With the press method, the mean oil value for the 27 samples was 18.37 per cent. The mean of the differences between duplicates was 0.75 ± 0.42. With the Soxhlet procedure, the mean oil value for all samples was 25.2 per cent, and the mean of the differences between duplicates was 0.33 ± 0.27.

The oil values by the press method were comparatively higher for the small-seeded samples than for the medium- and large-seeded samples (Table 4). This effect, while significant, was not considered a serious deterrent to the use of the method for plant breeders' samples. Selections with a medium level of oil content gave relatively higher oil values by the press method than did selections with either high or low oil content; but again the differences did not affect substantially the usefulness of the method.

RECOMMENDED PROCEDURE

Based on the results of these investigations, the following procedure is recommended:

Twenty whole seeds, typical of the sample, are weighed to the nearest milligram and spread uniformly on a double thickness of Whatman No. 1 filter paper (9-cm. circles). The filter paper and sample are placed on the platen of a Carver Laboratory Press and covered with two more circles of filter paper. A pressure load of 10,000 lb. is applied for 2 minutes, after which the pressed seeds are removed from the filter paper and weighed again. The loss in weight, representing the oil absorbed by the paper, is calculated as per cent oil in the original sample.

Oil values from duplicate pressings normally agree within 1 per cent. In heterogeneous samples, where greater differences are encountered, triplicate pressings are recommended.

One technician can analyse approximately 40 samples in duplicate in an 8-hour day.

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STEAM-OPERATED EQUIPMENT FOR THE PARTIAL STERILIZATION OF SOIL, FLATS AND POTS[1]

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ABSTRACT

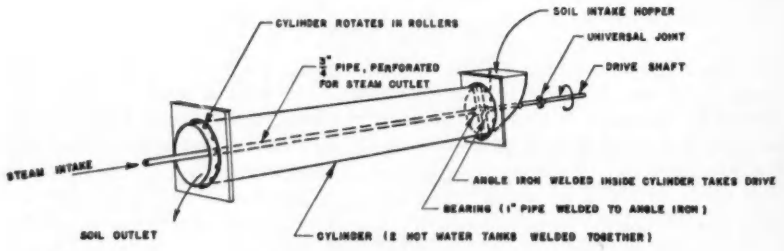
The equipment described consists of two units, with a common source of steam. In one unit, soil is sterilized by allowing soil particles to fall through steam in a rotating drum. Output is about 1 cubic yard of soil per hour. In the second unit, flats and pots are sterilized by 3 minutes' exposure to steam. Fungi and non-spore-forming types of bacteria are killed. The equipment is inexpensive and easily built.

INTRODUCTION

Equipment designed and placed in operation at Summerland, 4 years ago, has proved ideally suited for the steam pasteurization of soil, flats and pots at a research laboratory and in small commercial greenhouse establishments. The equipment is inexpensive, easy to operate, performs effectively and has very good capacity. The principle involved is based on that described by Newhall and Schroeder (1), wherein rapid sterilization is effected by allowing particles of soil to fall through heated air. Steam has been substituted for the dry heat employed in the Newhall and Schroeder machine, and additional modifications have been made. Pots and flats are pasteurized in a second unit supplied from the same steam source.

CONSTRUCTION OF SOIL PASTEURIZER

Figure 1 is a construction diagram, and Figures 2, 3, 4, and 5 are photographs of the completed apparatus. The steel cylinder (Figure 2,a) can be made from two discarded 40-Imperial gallon hot-water tanks,



SOIL STERILIZER – INNER VIEW

FIGURE 1. A diagram of the soil pasteurizer in longitudinal section.

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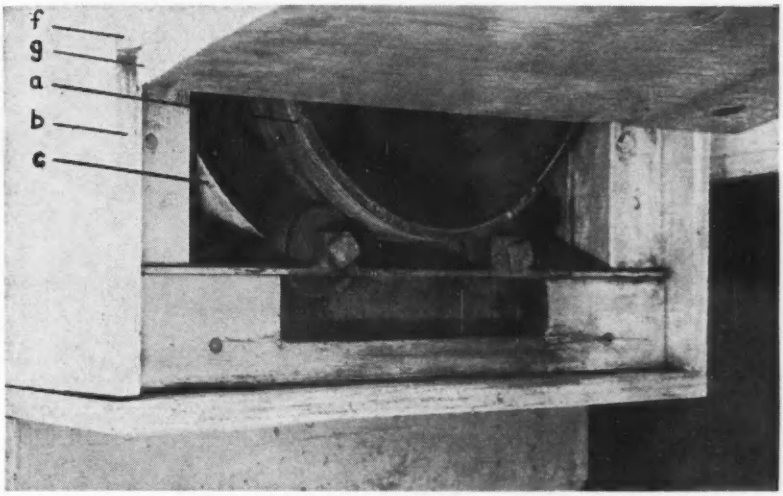

FIGURE 2. Discharge end of soil pasteurizer.

by cutting off both ends of each and welding two ends together. This makes a cylinder approximately 8 feet long and 14 inches in diameter. Alternatively, such a cylinder can be constructed from galvanized sheet steel 1/8 inch thick. The cylinder is enclosed in one end of a box (Figure 2,b) made of 3/4-inch fir plywood, 10 feet long and approximately 21 × 21 inches inside measurements. A casing of 24-gauge sheet steel, 17 inches in diameter (Figure 2,c) is inserted in the box to hold the insulation. A satisfactory insulating material is granulated vermiculite.

On the inner sides, near each end of the cylinder, wooden supports are installed to which rollers are bolted. The rollers, four at each end, serve as bearings on which the cylinder rotates. The end of the box, extending 2 feet beyond the cylinder, serves as a hopper (Figure 4,d) through which the soil enters the cylinder. No plywood is placed on the upper side of this portion. The end is sealed with 3/4-inch fir plywood. A sloping metal rim (Figure 4,e) is attached around the opening to guide the soil into the hopper. A piece of sheet metal (Figure 1) is slanted across the bottom of the hopper to facilitate movement of soil into the cylinder. A sliding gate, made of 18-gauge sheet steel, is fitted over the open end of the cylinder to regulate the rate of intake. A plywood platform is joined to the end of the box adjacent to the hopper, to carry the motor and gear box.

The discharge end of the box is sealed with a 3/4-inch plywood sheet (Figure 2,f), that is metal faced on the inside. Twenty-two gauge galvanized steel is satisfactory for this purpose. The lower portion of this plywood sheet, a strip 5 inches wide (Figure 2,g) is not fixed to the end of the box, but is hinged to the upper portion. It serves as an escape door for the soil as it is discharged from the machine, and helps to minimize loss of steam.

FIGURE 3. Plywood platform of soil pasteurizer, carrying electric motor, gear box, and universal joint assembly.

The box is supported on four legs, made of 2 × 6 inch lumber, at sufficient height above ground level to permit the placing of a wheelbarrow under the discharge end. The two legs at the discharge end are made adjustable, so that by lengthening or shortening them the desired rate of flow of soil through the cylinder can be obtained. The rate of soil flow governs the temperature to which the soil is subjected.

Power to rotate the cylinder is provided by a 1/2-h.p. electric motor and connected through a gear box, a universal joint, and a shaft that passes through the hopper (Figure 3). (A suitable joint is one used in a Model A Ford car). Rotation of the cylinder at 32 revolutions per minute has proved satisfactory. A strong angle iron (Figure 1) is welded

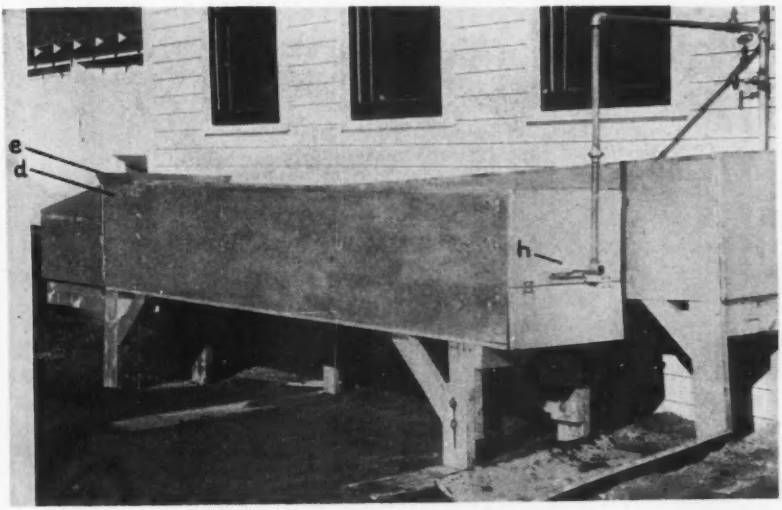

FIGURE 4. Assembled soil pasteurizer, photographed from discharge end; and pot and flat pasteurizer photographed from intake end.

across the diameter of the cylinder, approximately 1 inch from the intake end. This angle iron should be about 1½ inches wide by ⅛ inch thick, and should be fixed with the flat side at right-angles to the axis of the cylinder. It provides both an attachment with the gear box and a support for the end of a steam pipe, described below. On the hopper side of the bar, and at its centre, is welded a piece of ¾-inch threaded pipe about 3 inches long (Figure 1). This is coupled to a longer pipe that extends through the hopper and beyond the end of the box.

A ¾-inch pipe is used to distribute the steam in the cylinder. The pipe enters at the discharge end through the plywood end-plate (Figure 4,h) and passes along the axis the full length of the cylinder. It is supported at the hopper end in a cup made by welding a short piece of pipe to the centre of the crossbar (Figure 1). The connection of the steam pipe, outside the machine, to the source of supply should be flexible, allowing the discharge end to be raised or lowered without disconnecting the pipe. This can be done, if neccssary, by inserting a three elbow arrangement.

The steam for this machine is provided by a steam generator of the type used by garages for cleaning cars and trucks. The model used at Summerland develops a pressure of 80 lb. and has a reputed capacity of 80 gallons of water to steam per hour. In operation with this pasteurizer, the generator uses approximately 35 gallons per hour. Any steam boiler having a similar capacity should be suitable for use on this machine.

CONSTRUCTION OF POT AND FLAT PASTEURIZER

For little extra cost a unit can be added that gives efficient pasteurization of pots, flats, and other types of soil containers.

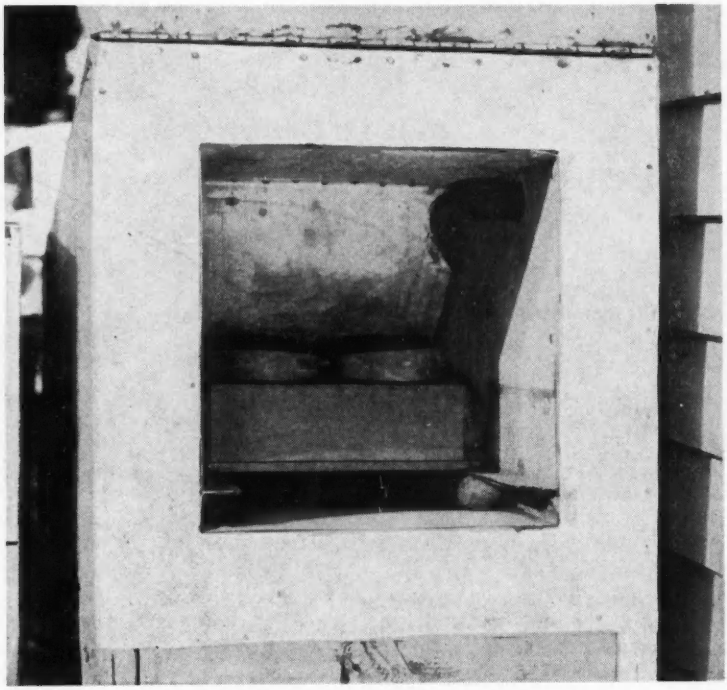

FIGURE 5. Intake end of flat and pot pasteurizer showing positions of metal slides and steam pipes.

This unit, shown in Figures 4 and 5, consists of a plywood box which encloses a rectangular metal inner case and is provided with steam from two pipes running from end to end.

The box is 16 feet long, built of $\frac{3}{4}$-inch fir plywood. Outside measurements are approximately 21 × 21 inches. The inner case, running the full length of the plywood box, is $13\frac{1}{2}$ inches wide, and is made of 24-gauge sheet steel. The space between box and inner case is packed with granulated vermiculite. The box is supported on 21-inch wooden legs.

The inner case is provided with metal slides at each side, $1\frac{1}{2}$ inches from the bottom, running the full length of the case. These can be inserted most readily by building the case in two parts, joined at the positions of the slides. The ends of each part are bent inward, and the bent flaps are soldered together to form the slides.

The steam pipes are brought in through the side of the box at the intake end. These are $\frac{3}{4}$-inch pipes, placed within the inner case at the upper and lower corners on one side. Holes $\frac{1}{4}$-inch in diameter are drilled 10 inches apart throughout the length of the case. All holes are placed so that steam is directed toward the centre of the chamber.

Each end of the box is enclosed by a door made of $\frac{3}{4}$-inch plywood, hinged at the top.

The dimensions quoted are for a pasteurizer to handle flats 12 inches wide. Obviously the width of the inner case should suit the size of flat which is pasteurized most frequently by the individual user. Pots can be conveyed on trays that have the same dimensions as these flats, but that have wire gauze substituted for the bottom slats.

OPERATION

The ingredients of the greenhouse soil required, i.e., the silt, sand, and peat or manure, are screened and placed in convenient piles near the hopper. The materials should be comparatively dry. Any desired combination of these materials can then be secured by introducing them, a pail or shovelful at a time, into the hopper. No pre-mixing is necessary as this is done very thoroughly as they pass through the cylinder. With the sliding gate fully closed, the hopper is filled and the steam turned into the cylinder. The steam should be allowed to flow for about 10 minutes to bring up the inside temperature and to permit the draining of excess moisture. The electric motor is then started and the sliding gate opened enough to give the desired rate of flow into the cylinder. Experience has shown that a pailful ($2\frac{1}{2}$ gal.) per minute is satisfactory. The legs on the discharge end are then adjusted to a height that allows the soil to pass through the cylinder in approximately 5 minutes. A drop of 6 inches over the length of the cylinder is the approximate amount required. Operated under the above conditions this machine pasteurizes about 1 cubic yard of material per hour, bringing it up to a temperature of 175-180°F.

In the second unit, flats and carriers are inserted at the intake end, the end doors are closed, and steam is released into the chamber for 3 minutes. A rod about 12 feet long is used to push the flats and carriers through to the discharge end.

TESTS OF PASTEURIZED MATERIALS

Samples of soil from several different batches were tested after treatment by plating on slants of potato dextrose agar. No colonies of organisms appeared for a period of 1 week, after which some bacterial growth appeared. These results suggest that the fungi and the non-spore-forming types of bacteria were killed and that the spore-forming kinds of bacteria survived. Flats of treated and untreated soil were seeded with lettuce, cucumber, and pepper to test the control of seedling diseases. No damping-off occurred in the flats of treated soil. Many plants were affected in the untreated soil.

Use of this equipment has permitted repeated use of materials and containers in which soil pathogens such as Verticillium had been cultured. Complete elimination of these organisms has been invariable.

ACKNOWLEDGEMENTS

Photographs were taken by S. R. Cannings. G. E. Woolliams conducted the tests of efficiency. A. S. F. Hanson and A. J. Baron assisted in construction of the original units.

REFERENCE

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A STUDY OF YIELD AND PROTEIN RESPONSE OF MALTING BARLEY VARIETIES TO DIFFERENT FERTILIZERS[1]

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ABSTRACT

A study was initiated in 1952 to determine the effect of various fertilizers on yield, protein percentage and saccharifying activity of three varieties of malting barley. The varieties Montcalm, O.A.C. 21 and Olli were used throughout a 6-year testing period from 1952 to 1957. During the first 4 years of experimentation, fertilizer treatments included 20 and 40 lb./acre of 11–48–0, 72 and 96 lb./acre of 16–20–0, and 60 lb./acre of ammonium nitrate. During the last 2 years of testing, the rates of fertilizer application consisted of 40, 60, 80 and 100 lb./acre of 11–48–0 and 96, 120 and 144 lb./acre of 16–20–0.

The combined analysis of variance for yield for each of the two periods (1952–55 and 1956–57) showed fertilizer effects to be significant to the 1 per cent point. A significant "variety × fertilizer" interaction was found to occur for each period and was attributable to the differential response of the three varieties particularly at the high levels of fertilizer application.

In 3 of the 5 years in which quality analyses were conducted, crude protein percentage and saccharifying activity of fertilized Montcalm and O.A.C. 21 were increased in comparison with control (unfertilized) material. In contrast, the protein percentage of Olli was not influenced by fertilizer treatment in any year.

Varietal fluctuations in saccharifying activity values, either between years or between treatments, paralleled those for protein.

From the practical viewpoint, it is doubtful whether the actual magnitude of protein increases, as found in the present study, was sufficient to consider fertilization as detrimental to malting quality.

INTRODUCTION

The relationship between the chemical composition of grain and the area of its production has received extensive study in the past. Annual surveys by the Board of Grain Commissioners of Canada (2) clearly indicate the influence of environment upon protein. Similarly, with the wide variety of chemical fertilizers now available for commercial use, numerous experimental programs have been outlined in an attempt to study, in terms of productivity and quality, the response of cereals to applications of nitrogenous fertilizer combinations.

Certain workers have reported an increased yield and protein content from grain to which nitrogenous fertilizers were applied (4, 9). Other investigators (6, 7, 8) have obtained increased yields with little or no effect on protein level.

It is generally known that, under natural conditions, a significant correlation exists between the rainfall in any particular area and the protein content of grain grown in that same area. It is conceivable, therefore, that the effects of nitrogenous fertilizers on the chemical composition of grain may also be modified by seasonal conditions. In 2 of the 3 years of investigation, Geddes *et al.* (3) obtained an increase in protein content in

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fertilized Reward wheat. During the third season, below-normal rainfall resulted in a general increase in protein content, thereby minimizing or masking any effect attributable to fertilizers.

Response to fertilizer has also been shown to be modified, depending upon whether treatments were applied to fallow crops or to stubble crops. When nitrogenous fertilizer top dressings were applied to barley grown on summerfallow, Hopkins (5) obtained a significant increase in protein content but no material increase in yield. The opposite effect was obtained, however, when identical treatments were applied to the same varieties grown on stubble.

The fact that fertilizers under certain conditions can modify the chemical composition of grain is important from the standpoint of its commercial value. High-protein bread wheats are essential to the maintenance of the Canadian export market. A high protein content in malting barley, however, is a very undesirable factor. It is conceivable, therefore, that, in those regions suited for the production of malting barleys, the use of high rates of nitrogenous fertilizers to increase yields could reduce the quality of this grain destined for malting purposes.

The present study was initiated in 1952 by J. B. Harrington* for purposes of determining yield and quality response of three malting barley varieties when treated with varying rates of commercial fertilizers. Results obtained over a 6-year period are reported.

MATERIALS AND METHODS

The test site chosen for this investigation was located on Tisdale clay soil situated in one of the major malting areas of northeastern Saskatchewan. The test was conducted on summerfallow each year and was arranged as a split-plot design. The three malting barley varieties, Montcalm, O.A.C. 21 and Olli, were included in the test each year. For the period, 1952-1955 inclusive, each variety was replicated three times and received the following rates of the type of fertilizer indicated:

Check (unfertilized)
20 lb. of 11–48–0
40 lb. of 11–48–0
72 lb. of 16–20–0
96 lb. of 16–20–0
60 lb. of ammonium nitrate.

In 1956 and 1957, the tests were increased to six replicates. In addition, the ammonium nitrate treatment was excluded and the rates of 11–48–0 and 16–20–0 fertilizers were increased to include the following:

Check (unfertilized)
40 lb. of 11–40–0
60 lb. of 11–48–0
80 lb. of 11–48–0
100 lb. of 11–48–0
96 lb. of 16–20–0
120 lb. of 16–20–0
144 lb. of 16–20–0.

*At that time Professor and Head of the Field Husbandry Department, University of Saskatchewan.

All fertilizers were sown with the seed.

Yields resulting from each treatment were determined on the basis of threshed grain harvested from the two centre rows of a four-rowed plot, 12 feet in length. Split-plot analyses of variance were calculated on the basis of results of individual years, as well as from the combined data for the two periods, 1952-55 and 1956-57 inclusive.

Crude protein percentages (determined on a dry basis) and saccharifying activity (measured in degrees Lintner) were determined, using the method as outlined by Bendelow *et al.* (1). Material for both chemical analyses consisted of a 160-gram sample of grain drawn from a treatment-variety bulk. Since it was not possible to measure replicate variance for either protein or saccharifying activity, second-order interactions were used to test treatment differences.

RESULTS

Yield Response (1952-55)

The mean yield of the three varieties receiving each fertilizer treatment for the combined 4-year period is shown in Table 1, while the analysis of variance for yield appears in Table 2.

As shown in the combined analysis of variance, differences between treatments were highly significant. All levels of fertilizer application resulted in an increased mean yield, ranging from an average of 2.4 bushels per acre as a result of the 60-lb. treatment of ammonium nitrate, to 5.4 bushels per acre from the 72-lb. treatment of 16-20-0. Application of 16-20-0 at 96 lb. per acre, however, resulted in an average yield response no greater than that obtained from the 72-lb. treatment. The danger of misinterpreting fertilizer responses, based upon too few years of experimentation, was clearly illustrated by data obtained in different years. In 2 of the 4 years, the highest rate of 16–20–0 (96 lb./acre) actually decreased the mean yield slightly below that obtained from the lowest application rate of this same fertilizer (72 lb./acre). Similarly, in 1954, the highest treatment of 11-48-0 depressed yields below that obtained from the 20-lb.

TABLE 1.—MEAN YIELDS IN BUSHELS PER ACRE OF EACH OF THREE VARIETIES OF BARLEY RECEIVING FERTILIZER FOR THE 4-YEAR PERIOD, 1952–1955

Treatment	Variety			Mean yield for treatments
	Montcalm	O.A.C. 21	Olli	
Check	43.9	45.0	45.2	44.7
20 lb. 11–48–0	46.0	47.9	47.8	47.2
40 lb. 11–48–0	47.0	51.5	48.4	49.0
72 lb. 16–20–0	47.9	52.6	49.7	50.1
96 lb. 16–20–0	49.0	50.2	50.4	49.9
60 lb. ammonium nitrate	49.1	46.7	45.4	47.1
Mean yield for varieties	47.2	48.8	47.8	
L.S.D. (P = .05)				1.6

TABLE 2.—PARTIAL ANALYSIS OF VARIANCE FOR YIELD RESPONSE TO FERTILIZERS, 1952–1955

Variation due to	D. F.	M. S.	F Value	5%	1%
Main plots	35				
Fertilizers	5	15096	13.49**	2.30	3.21
Varieties-fertilizers	10	2787	2.49*	1.94	2.53
Years-fertilizers	15	4411	3.94*	1.85	2.37
Varieties-years-fertilizers	30	13910	12.43**	1.63	1.98
Error (b)	120	1119	—		

TABLE 3.—MEAN YIELDS IN BUSHELS PER ACRE OF EACH OF THREE VARIETIES OF BARLEY RECEIVING FERTILIZER FOR THE 2-YEAR PERIOD, 1956–1957

Treatment	Variety			Mean yield for treatments
	Montcalm	O.A.C. 21	Olli	
Check	40.4	47.7	39.6	42.5
40 lb. 11–48–0	58.0	52.1	54.1	54.7
60 lb. 11–48–0	65.1	55.6	55.7	58.8
80 lb. 11–48–0	66.7	54.8	58.8	60.1
100 lb. 11–48–0	63.6	62.9	58.2	61.5
96 lb. 16–20–0	59.5	57.3	53.5	56.8
120 lb. 16–20–0	63.8	58.7	56.4	59.6
144 lb. 16–20–0	64.3	59.7	55.5	59.8
Mean yield for varieties	60.2	56.1	54.0	
L.S.D. (P = .05)				3.7

treatment. These differences, however, were not statistically significant. As might be expected on the basis of these yearly fluctuations, a significant "year-fertilizer" interaction was obtained as illustrated in the analysis of variance, Table 2.

As borne out by the mean yields for treatments in Table 1, the return per acre was not proportional to the total amount of nitrogen applied but rather appeared dependent upon a balanced N/P relationship. The application of 60 lb. of ammonium nitrate, for example, was not as effective in terms of the actual amount of nitrogen applied as when both nitrogen and phosphorus were used in the form of 11–48–0 or 16–20–0.

Yield Response (1956-57)

There was some indication from the 1952-55 results that the yielding potential of varieties was reaching a threshold above which further increase in fertilizer application had little or no effect. The mean yield responses of the three varieties treated with both 11–48–0 and 16–20–0, at rates extended beyond those used in the 1952-55 period, are shown in Table 3. The combined analysis of variance is presented in Table 4.

Although fertilizer application in 1956 increased yields substantially over those obtained from the control (untreated) plots, the lowest rate of

TABLE 4.—PARTIAL ANALYSIS OF VARIANCE FOR YIELD RESPONSE TO FERTILIZERS, 1956–1957

Variation due to	D. F.	M. S.	F Value	5%	1%
Main plots	35				
Fertilizers	7	134794	21.43**	2.06	2.74
Varieties-fertilizers	14	11519	1.83*	1.80	2.28
Years-fertilizers	7	67107	10.67**	2.06	2.74
Varieties-years-fertilizers	14	9479	1.51	1.80	2.28
Error (b)	210	6291	—		

application of any particular fertilizer resulted in essentially the same yield as obtained from the highest rate. The threshold effect which was suggested from the 1952-55 data, therefore, appeared to be operative again in 1956. Over-all yield differences attributable to fertilizers, however, were slightly below the point of statistical significance.

In 1957, however, an entirely different response was obtained. All fertilizer rates, with the exception of the highest application of 16–20–0 (144 lb./acre) resulted in substantial yield increases. Fertilizer effects for that particular year were significant to the 1 per cent point. The two fertilizer types also reacted differently in each of the 2 years, in that in 1956 the 16–20–0 formulation resulted in the highest yields, while, in 1957, 11–48–0 was in general most effective. In line with the results obtained in the 4 previous years' experimentation, the combined analysis of variance for yield in the 1956 and 1957 tests showed that the effect of fertilizers as well as the "year-fertilizer" interaction were highly significant.

It is interesting to note that a significant "variety-fertilizer" interaction was obtained in both the 1952-55 and 1956-57 yield analyses. In order to determine individual cross-differences responsible for the significant interaction, comparisons were made between varieties with respect to the occurrence of a differential yield response to each fertilizer treatment. In the 4-year period, 1952-55, O.A.C. 21 showed the greatest response to all fertilizer treatments with the exception of the 60-lb. application of ammonium nitrate. This treatment reversed the order of varietal response in that Montcalm exhibited the greatest yield increase, while Olli responded least.

In the 1956-57 experiment, the pattern was similar to that found in the preceding 4-year period. Intervarietal comparisons showed that the mean yield increase of O.A.C. 21 was significantly greater than that for Olli at all fertilizer levels. A significant decrease in mean yield of Montcalm occurred at the 100-lb. treatment of 11–48–0, as compared with a positive response of O.A.C. 21 to this same treatment.

Quality Response

The two components, crude protein percentage and saccharifying activity, were used as a measure of quality of malting barley as affected by fertilizers. Quality data obtained from the 1952 experiment were considered unreliable; consequently only 5 years' results are reported. In that two distinct levels of fertilizer applications were used at different

times during the 5-year period, results obtained from the two periods of experimentation are reported separately in accordance with the manner of presentation of yield effects. Tables 5 and 6 show the crude protein percentages and saccharifying activity (°L) respectively, obtained in each of the three varieties for the 1953-55 period.

It may be noted that in the 2 years, 1953 and 1954, values obtained from each of the three varieties with respect to protein and saccharifying activity were relatively constant for all fertilizer treatments. In 1955, however, both Montcalm and O.A.C. 21 responded in such a way that their protein levels were increased with all fertilizer treatments, with the exception of ammonium nitrate. In comparison with the controls (unfertilized plots), the range of increase of protein percentage of fertilized Montcalm and O.A.C. 21 was from 1.2 to 2.1 per cent and 0.4 to 1.0 per cent respectively. From Table 7 it may be seen that the trend toward increased

TABLE 5.—CRUDE PROTEIN PERCENTAGES (DRY BASIS) OF THREE MALTING BARLEY VARIETIES FOLLOWING FERTILIZER TREATMENTS, 1953–55

	Variety									
	O.A.C. 21			Montcalm			Olli			
	Years									
Treatment	1953	1954	1955	1953	1954	1955	1953	1954	1955	Treatment mean*
Check	13.0	15.5	16.4	13.2	15.2	14.6	12.7	16.4	16.5	14.9
20 lb. 11–48–0	12.8	15.0	17.4	12.8	14.7	16.6	13.0	16.2	16.3	15.0
40 lb. 11–48–0	13.0	15.5	17.1	13.1	15.2	15.8	13.2	16.5	16.4	15.1
72 lb. 16–20–0	13.1	15.4	17.3	13.1	15.1	16.7	13.6	16.3	16.8	15.3
96 lb. 16–20–0	12.7	15.8	16.8	12.9	14.8	16.3	13.2	16.8	16.7	15.2
60 lb. ammonium nitrate	13.6	15.7	16.0	13.8	15.0	14.4	12.6	15.9	16.8	14.9

*No significant difference found

TABLE 6.—SACCHARIFYING ACTIVITY (°L) OF THREE MALTING BARLEY VARIETIES FOLLOWING FERTILIZER TREATMENTS, 1953–55

	Variety									
	O.A.C. 21			Montcalm			Olli			
	Years									
Treatment	1953	1954	1955	1953	1954	1955	1953	1954	1955	Treatment mean*
Check	204	253	282	220	257	272	238	278	309	257.0
20 lb. 11–48–0	217	234	297	221	251	326	240	281	299	262.9
40 lb. 11–48–0	218	237	290	214	262	293	264	295	303	264.0
72 lb. 11–48–0	222	237	303	226	256	320	246	289	208	267.4
96 lb. 11–48–0	219	237	326	206	259	313	257	289	326	270.2
60 lb. ammonium nitrate	228	246	283	225	254	272	233	276	327	260.4

*No significant difference found

protein in these same two varieties also holds true for the 1956 and 1957 experiments. The maximum increase of crude protein percentage in Montcalm and O.A.C. 21 in 1956 was 2.0 and 2.2 per cent respectively. In 1957 the 96-lb. application of 16–20–0 appeared most effective by increasing protein in these same two varieties by 1.9 per cent. Although there appeared to be no general relationship between rates of fertilizer and response in protein levels for these two varieties, the data suggest that the low rate of both 11–48–0 and 16–20–0 affected protein levels more than the higher rates of application.

It is of interest to note the differential in varietal protein response obtained from fertilizers. In contrast to the behaviour of Montcalm and O.A.C. 21, the protein level of Olli remained unaffected by all fertilizer treatments (Tables 5 and 7). This contrast in varietal reaction was responsible for the highly significant "variety-fertilizer" interaction obtained in the 1956–57 combined analysis of variance for protein (Table 8). An analysis of cross differences of protein percentages at all fertilizer levels, in comparison with the appropriate check, revealed that differences in response between Montcalm and O.A.C. 21 were not significant. Cross differences between O.A.C. 21 and Olli, however, were highly significant

TABLE 7.—CRUDE PROTEIN PERCENTAGES (DRY BASIS) OF THREE MALTING BARLEY VARIETIES FOLLOWING FERTILIZER TREATMENTS, 1956–57

	Variety						
	O.A.C. 21		Montcalm		Olli		
	Years						
Treatment	1956	1957	1956	1957	1956	1957	Treatment mean*
Check	13.8	15.0	13.9	13.2	14.6	16.5	14.5
40 lb. 11–48–0	15.4	16.7	14.5	14.8	13.7	16.6	15.3
60 lb. 11–48–0	15.5	16.3	14.7	14.8	13.7	16.6	15.3
80 lb. 11–48–0	15.6	15.8	13.5	14.5	14.2	16.2	15.0
100 lb. 11–48–0	15.0	15.9	14.7	14.1	14.8	16.3	15.1
96 lb. 16–20–0	15.8	16.9	14.6	15.1	13.9	16.7	15.5
120 lb. 16–20–0	16.0	16.7	14.9	14.9	14.0	16.3	15.5
144 lb. 16–20–0	15.7	16.6	14.6	14.8	14.0	15.9	15.3

*No significant difference found

TABLE 8.—PARTIAL ANALYSIS OF VARIANCE FOR PROTEIN RESPONSE TO FERTILIZERS, 1956-57

Variation due to	D. F.	M. S.	F Value	5%	1%
Fertilizers	7	0.64	1.68	2.78	4.30
Varieties-fertilizers	14	0.38	3.92**	2.51	3.78
Years-fertilizers	7	0.13	1.34	2.78	4.30
Varieties-years-fertilizers	14	0.097	—		

TABLE 9.—PARTIAL ANALYSIS OF VARIANCE FOR PROTEIN OF THREE MALTING BARLEY VARIETIES FOLLOWING FERTILIZER TREATMENTS COMMON TO THE 5-YEAR PERIOD, 1953-57

Variation due to	D. F.	M. S.	F Value	5%	1%
Fertilizers	2	1.51	6.46**	3.63	6.23
Varieties-fertilizers	4	0.35	1.50	3.01	4.77
Years-fertilizers	8	0.23	0.99	2.59	3.89
Varieties-years-fertilizers	16	0.23	—		

for all levels of fertilizer. Similar results were obtained for differences between the protein responses of Montcalm and Olli with the exception of the 80 and 100-lb. treatments of 11–48–0. At these two levels of treatment, differences in protein response between Montcalm and Olli were slightly below the 5 per cent level of significance. As a result of the masking influence attributable to this one variety (Olli), it was not possible to demonstrate an over-all statistically significant effect of fertilizer on protein percentage.

The pattern of fertilizer effect on saccharifying activity closely corresponded to that for protein. For the 3-year period during which it was possible to demonstrate an increased protein content in the varieties Montcalm and O.A.C. 21 (1955-57 inclusively), the saccharifying activity of these varieties also increased. The variety Olli, however, again reacted in a contrasting manner to the other two varieties. Fertilizer effects on saccharifying activity were non-significant when the data on the three varieties were analysed together.

It was possible to compare two fertilizer treatments with controls for the entire 5-year period during which quality data were analysed. These treatments were the 40 lb./acre application of 11–48–0 and the 96 lb./acre rate of 16–20–0. The analyses of variance for protein only is shown in Table 9.

It is seen that the effects of fertilizer on protein levels were significant to the 1 per cent point. Although not shown in the present results, analysis of variance for saccharifying activity during the same 5-year period was significant to the 5 per cent point, thus illustrating again the close relationship between this character and protein level. It is recognized that the result of this particular analysis lose some of their significance because of the reduction in degrees of freedom compared with previous analyses in which all fertilizer rates were included. It is felt, however, that the trend toward increased protein and saccharifying activity is real and points out the danger of formulating conclusions regarding fertilizer responses based upon too few years of experimentation. The fact that the effects of "years" on yield returns and components of quality were significant in many of the analyses reported herein also points out the variation in fertilizer effect that may be expected due to varying climatic conditions from year to year.

DISCUSSION

The three malting varieties chosen for the present study are currently grown in Western Canada and vary in popularity according to the area and conditions under which they are grown. The variety O.A.C. 21 is considered the official standard with respect to Canadian malting requirements. The acreage sown to this variety, however, has gradually decreased since 1945, at which time Montcalm was licensed for sale in Canada. Because of its superior straw and neck strength, as well as its smooth-awned character, Montcalm has largely replaced the older malting variety O.A.C. 21. The variety Olli is a relatively low yielding, weak-strawed variety in relation to Montcalm. Because of its early maturity, however, Olli has a place in areas where delayed seeding is necessary but which are suitable for the production of a malting barley.

Although eligible as a malting barley, Olli generally exhibits a consistently higher protein level than either Montcalm or O.A.C. 21. Considering only the unfertilized plots of Olli in comparison with those of Montcalm, the range of differences in protein values obtained in the present study was from 0.4 to 3.3 per cent in favour of Olli. The over-all mean difference was 1.0 per cent. The range of differences between Olli and O.A.C. 21 was considerably less, with the mean difference being only 0.2 per cent. It is difficult, therefore, to explain the reason for the differential protein response to fertilizer between Olli and the other two varieties. If an initially high protein percentage in any variety had the effect of masking the effect of nitrogenous fertilizer in further raising this level, O.A.C. 21 should have behaved similarly to Olli. As shown in Table 7, however, protein levels of O.A.C. 21 responded in a manner similar to those of Montcalm under the influence of fertilizers.

It is generally true that the diastatic activity is very closely and positively associated with the protein percentage. Saccharifying activity of Olli in the present study was considerably higher than that of O.A.C. 21 or Montcalm for all years, which was in accordance with the comparatively higher protein level during the same period of time. In addition, with the exception of one year, the ranking of the three varieties with respect to protein values of unfertilized grain parallels the ranking of its diastatic values. This comparison also emphazises the very close relationship between these two components of quality.

The fact that nitrogen alone is not the limiting factor in yield in the area in which the study was conducted was evident from the low efficiency of ammonium nitrate in terms of the actual amount of nitrogen/acre applied and the net increase in yield/acre. More interesting, however, is the apparent lack of protein response to the comparatively high rate of application of nitrogen in the form of ammonium nitrate. In 1955, when the protein content of both Montcalm and O.A.C. 21 was increased by a maximum of 2.1 per cent and 1.0 per cent respectively in response to certain rates of application of ammonium phosphate fertilizers, the protein level in grain samples from ammonium nitrate-treated plots was comparable to that of controls. It was because of this obvious lack of quality response from ammonium nitrate that this particular treatment was deleted from the experiment after 4 years of testing.

The question remains, however, as to how crucial from the point of view of the malting trade are the increases in crude protein resulting from fertilizer as found in the present study. The variation in protein and saccharifying activity from year to year for any of the three varieties, or between varieties for any one year, was more than the greatest increase obtained as a result of fertilizers. Considering only unfertilized plots, the maximum range within any one variety over the 5 years was 3.8 per cent, while a difference as great as 3.3 per cent was found between varieties in 1957. Compared with these inherent or environmental fluctuations, the greatest protein increase attributable to fertilizers was 2.2 per cent (120-lb. application of 16–20–0 to O.A.C. 21 in 1956). The importance of this increase from the point of view of fertilizer application being harmful to malting quality, therefore, appears to be of questionable significance. In the present study, the beneficial effects of fertilizers as measured by response in yields and maturity were far more important than any possible detrimental influence that they may have had on malting quality.

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THE BULK BIN METHOD OF HANDLING FRUIT.
I. COOLING AND BRUISING OF McINTOSH APPLES[1]

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ABSTRACT

Experiments were conducted to compare the rate of cooling and amount of bruising and stem puncturing of apples handled in 25-bushel bulk bins, and in conventional bushel boxes.

Fruit in bins with no facilities for ventilation cooled more slowly than in bushel boxes; fruit in bins with a 1-inch opening on all sides of the bin, at floor level, cooled almost as quickly as in bushel boxes.

McIntosh apples in good condition, dumped from the containers soon after being picked, suffered less bruising when handled in bins than when handled in boxes. There was no difference in the amount of stem puncturing caused by the two methods. When the apples were stored for 7 days without refrigeration before being dumped from the containers, there was no difference between the methods in the occurrence of bruising but there were more stem punctures in the bin-handled fruit. When the apples were held in cold storage for 36 days before being dumped, there was no difference between the methods, in either bruising or stem puncturing.

INTRODUCTION

The bulk bin method of handling fruit originated in New Zealand in 1953. It was used in Michigan in 1955 for handling fruit for processing plants (1) and in 1956 for handling fruit destined for the fresh fruit market (2).

FIGURE 1. Orchard trailer used for transporting 25-bushel bulk bins.

[1] Joint contribution from the Field Husbandry, Soils and Agricultural Engineering Division, and No. 934 from the Horticulture Division, Experimental Farms Service, Canada Department of Agriculture, Ottawa, Ont.
[2] Agricultural Engineer.
[3] Pomologist.

FIGURE 2. Tractor carrying a 25-bushel bulk bin on a simple lift mounted on the three-point hitch.

In 1957, over 4,000 25-bushel bins were used for harvesting apples and pears in the Okanagan Valley of British Columbia. The bins were 48 inches long, 43 inches wide and 24 inches deep. The height of the bins, including integral pallet, was 29 inches. The sides and bottoms were made of exterior grade plywood.

Bins were transported in the orchard on a trailer or with an inexpensive lift mounted on the 3-point hitch of a tractor (Figures 1 and 2). Hauling from orchard to packing-house was done mostly with trucks or trailers. At the packing-house bins were transported with regular fork-lift equipment.

Two systems were used for dumping the fruit from the bins. In the "inversion system", the full bins were inverted slowly by a mechanical dumper and the fruit was gently released on to a wide belt; in the "end-gate system", one end of the bin was elevated to a suitable height and the fruit flowed through a gate on the other end on to a wide belt.

Since this was the first year such containers were used in the British Columbia fruit industry, it was desirable to determine whether fruit handled in the bulk bins could be cooled as quickly as in conventional bushel boxes, and also whether fruit handled in bulk bins suffered more bruising and stem puncturing than fruit handled in the bushel boxes.

EXPERIMENTS ON RATE OF COOLING OF FRUIT

Procedure

The cooling rate of apples in bulk bins was compared with that in bushel boxes in squeeze-truck loads. (A squeeze-truck load is a compact stack of 36 boxes in 6 tiers of 6 boxes each). The bins used in the experiments were provided with ventilation as shown in Table 1. The experiment was replicated four times.

TABLE 1.—MEAN COOLING RATES[1] OF APPLES IN BULK BINS AND BUSHEL BOXES

Container	Square inches of ventilation provided			Mean cooling rate[2]
	In sides at floor level	In bottom	Total	
Bushel box	—	—	—	0.04425
Bin type 1	0	0	0	0.02350
2	48	0	48[3]	0.03075
3	48	0	48	0.02825
4	48	27	75	0.03975
5	48	40	88	0.05700
6	118	0	118	0.03750
7	118	27	145	0.04400
8	118	40	158	0.04600

[1] Expressed as $\frac{\text{Fruit temperature reduction}}{\text{(Hours) (Average temperature difference between fruit and air)}}$.
[2] LSD in mean cooling rates = ± 0.01808 at 5% level.
[3] This bin was lined with corrugated cardboard on sides and bottom.

For each experiment, 8 bins (one of each type) and 3 squeeze-truck loads (108 bushel boxes) of apples were picked on the same day and placed in cold storage during the evening of that day. The bins were stacked 6 high, and the boxes 3 squeeze-truck loads, or 18 boxes, high. Bins and boxes were flanked on all sides by other bins or boxes.

As the fruit was being placed in the cold storage a calibrated thermistor (thermal resistor) with long copper leads was placed in the centre of each bin and in the centre of the bushel box nearest the centre of the 3 squeeze-truck loads. Another thermistor was used to measure air temperature. The copper leads were brought to a panel for convenient reading of the resistances of the thermistors with a Wheatstone bridge. Resistance readings were made at approximately 12-hour intervals until the fruit temperature in all containers was nearly the same as the room temperature. The cooling rate for each container was calculated by the method of Pentzer, Perry *et al.* (3):

$$\text{Cooling rate} = \frac{\text{Total fruit temperature reduction in degrees F}}{\text{(Hours) (Average temperature difference in degrees F)}}$$

where the average temperature difference is the average of the temperature differences between fruit and air at the times of the various periodic readings.

Results and Discussion

The mean cooling rates of apples in bins and boxes are presented in Table 1.

There was a difference (at the 5 per cent level) between the cooling rates of the bushel box and bin type *1*, in which no ventilation was provided. No other differences between bins and the bushel box were significant at this level. There were differences between the bins: bins *5* and *8* cooled more rapidly than bins *1* and *3;* bin *7* cooled more rapidly than bin *1*.

The mean cooling rate determined for bin type *5* was higher than would be expected. No explanation can be offered as to why this type cooled more rapidly than type *8*, which had more ventilation in the sides and the same amount of ventilation in the bottom.

In the course of the experiments it was found that bottom slots greatly weakened the bins. Therefore, it is considered desirable to provide all necessary ventilation in the sides, at the floor line if possible. To avoid damage to fruit, the depth of the openings should be limited to the radius of the smallest fruit handled, or to approximately 1 inch.

Considering both bin strength and cooling rate, bin type *6*, in which a 1-inch opening was provided most of the way around the bin at floor level, and in which no bottom slots were provided, appears to be the most satisfactory of the bin types used in the experiments.

EXPERIMENTS ON BRUISING OF FRUIT

Procedure

Four experiments were conducted to compare the bruising and the stem puncturing that occurred in fruit handled in bulk bins and in bushel boxes. In all experiments, McIntosh apples were used because they are very susceptible to both bruising and stem puncturing. For each experiment, 5 bulk bins and 125 bushel boxes were picked in the same orchard by the same pickers on the same day.

In the *first* experiment, the fruit was hauled from orchard to packing-house on a trailer over a paved road. The bins were dumped by the end-gate method 24 hours after being picked. The bushel boxes were dumped by hand at the same time.

In the *second* experiment, the fruit was hauled from orchard to packing-house over a paved road by means of a tractor-mounted fork-lift. The bins were dumped by the inversion method 24 hours after being picked. The bushel boxes were dumped by hand at the same time.

In the *third* experiment, the fruit was hauled to the packing-house over a paved road by means of a tractor-mounted fork-lift 24 hours after being picked and was then held in common storage for 7 days before being dumped. The bins were dumped by the inversion method and the bushel boxes by hand.

In the *fourth* experiment, the fruit was hauled on a trailer over a rough gravel road the same day on which it was picked. It was held in cold storage for 36 days before being dumped. The bins were dumped by the inversion method, and the bushel boxes by a mechanical dumper.

In each experiment, fruit samples were taken at random from the belt carrying the fruit to the wiper. A sample consisted of 125 fruits per bin and 5 fruits per bushel box. The samples were placed in papier-maché trays so that each apple was completely separated from the others. This ensured that no further bruising or stem puncturing would occur before the samples were examined. The samples were placed in cold storage for several days and then a careful count was made of all bruises and stem punctures on each apple. Bruises were classified according to diameter. Bruises under ½ inch were given a score of *1;* those between ½ inch and 1 inch a score of *2;* and those over 1 inch a score of *3*.

TABLE 2.—COMPARISON OF BRUISING AND STEM PUNCTURING ON McINTOSH APPLES HANDLED IN BULK BINS AND BUSHEL BOXES

Experiment	Fruit pressure at time of dumping[1]	Mean bruise score per apple		Mean stem punctures per apple	
		Bins	Boxes	Bins	Boxes
1	14.5	2.1	4.1[2]	0.059	0.093
2	15.4	1.8	3.8[2]	0.122	0.083
3	13.6	4.8	4.1	0.139[2]	0.080
4	12.1	5.9	5.3	0.282	0.242

[1] Measured with a Ballauf pressure tester
[2] Significant at 1% level

RESULTS AND DISCUSSION

A summary of bruise scores and stem punctures is presented in Table 2. This shows that, in Experiments *1* and *2*, where the apples were in good initial condition, and were dumped soon after being picked, there was only half as much bruising in apples handled in the bulk bins as in those handled in bushel boxes, but there was no difference (at the 1 per cent level) between the methods in the occurrence of stem punctures.

In Experiments *3* and *4*, where the apples were stored 7 days in common storage, or 36 days in cold storage, before being dumped, there was no difference (at the 1 per cent level) between the methods in the occurrence of bruises. In Experiment *3*, however, there was more stem puncturing in bins than in boxes.

Evidently, McIntosh apples handled in the new 25-bushel bulk bin are not likely to suffer any more bruising or stem puncturing than when handled in the conventional bushel box.

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ANTHOCYANINS AND ANTHOCYANIDINS OF THE BARLEY PERICARP AND ALEURONE TISSUES

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ABSTRACT

The anthocyanins and anthocyanidins of the pericarp and aleurone tissues of one white, three blue, two purple, and two black barley varieties were isolated by paper chromatography. Two anthocyanins, "B" and "C" (perhaps cyanidin-3-glucoside), occurred in one black and in the blue and purple varieties. Additionally, of three anthocyanins found in the two purple varieties, two, "D" and "E", were common to both and one, "F", was found only in the variety Gopal. Also found in the purple varieties was a poorly resolved group of anthocyanins designated as "A". Two anthocyanidins, delphinidin and cyanidin, were found in all varieties and one, pelargonidin, was found only in the purple varieties. It is probable that anthocyanins A and A_1 are delphinidin derivatives; anthocyanins C and D, cyanidin derivatives; and anthocyanins E and F, pelargonidin derivatives. The relationships of the anthocyanins to colour inheritance patterns were discussed.

Colour may develop independently in the endosperm, aleurone, pericarp, and chaff of the barley grain (11, 17). The grain at maturity may be black, blue, purple, red, yellow, grey, white, or an intergrade of these. The inheritance of the principal colour genes has been reviewed by Smith (17). The expression of the colour genes may be modified considerably by variations in climate and soil (7, 15, 18) and may occur late in development of the plant (8). Inheritance in pericarp and chaff is, of course, maternal and diploid; in aleurone and endosperm, triploid and maternal-paternal. Colour in pericarp may mask colour in inner tissues. The pigments largely responsible for grain colours are flavonoid; purple, blue, and red are given by anthocyanins (7, 8, 4, 1) and other flavonoids give brown and yellow and act as co-pigments of anthocyanins. Melanin-like pigments occur in black and possibly in brown and grey grains (7, 4, 1).

Colour in barley is useful, superficially, to distinguish one variety from another (1, 5) and may serve as a hallmark of quality, viz., Canadian malting barley is commonly blue. A more fundamental role in feed and malt quality has been suggested (6, 10, 16) for the barley pigments. The present study, restricted though it is to anthocyanins and anthocyanidins of the pericarp and aleurone, was undertaken to provide a chemical basis for studies of barley colour inheritance and, secondly, to further biogenetic and quality studies.

MATERIALS

Eight barley varieties were selected from fifty-seven immediately available for pigment extraction, viz., the black barley varieties, Lion and Gatami; the purple, Black Hulless and Gopal; the blue, Kwan, Mont-

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calm, and Trebi; and the white, Golden Pheasant. The varieties were chosen for the uniformity of their colour development in the University plots. The seed stocks of the varieties Gopal and Golden Pheasant were given to the authors by G. A. Wiebe, Bureau of Plant Industry, United States Department of Agriculture; the seed stocks of the other varieties were provided by D. G. Hamilton, Chief, Cereal Division, Canada Department of Agriculture, Ottawa.

METHODS

(*a*) *Extraction of Anthocyanins*

To obtain material with as much aleurone and pericarp tissue as possible, barley grains were pearled well into the endosperm. Five to eight grams of the powder from the pearling operation were collected, scanned with a magnet to remove any metallic bristles, and 1 per cent hydrochloric acid was added at the rate of 8 to 10 ml. for each gram of dust. The material was then extracted in a 10KC Raytheon sonic oscillator for 20 to 30 minutes, withdrawn with a pro-pipette, and centrifuged in polythene tubes at 14,000 r.p.m. for 5 to 7 minutes. The centrifugate was re-extracted with 1 per cent hydrochloric acid and again centrifuged. The combined supernatants were placed in the refrigerator overnight. After thawing, a proteinaceous coagulum formed and was removed by centrifuging at 14,000 r.p.m. for 5 to 7 minutes. Volume was reduced *in vacuo* at 30°C. and further centrifugation, freezing, and volume reduction was undertaken as needed to obtain a clear anthocyanin-containing extract of 1 or 2 ml. volume. Ethyl acetate (free from ethanol and acetic acid), added slowly and in quantity, was vigorously shaken with the extract. The mixture was allowed to stand for 10 minutes. The ethyl acetate removed water and some interfering substances from the anthocyanin extract. Repeated scrubbing with ethyl acetate reduced the pigment extract to 0.2 to 0.3 ml. Ethyl acetate remaining in the extract was removed in benzene.

In some instances, where the anthocyanin concentrations in aleurone and pericarp were low, rapid extraction was obtained with 1 per cent hydrochloric acid in methyl alcohol rather than in water. After extraction with the methanolic solvent, volume was reduced *in vacuo* to about 10 ml. and then about 20 ml. of 1 per cent aqueous hydrochloric acid was added. Ten minutes' centrifugation at 14,000 r.p.m. then brought down a large amount of colloidal material which was discarded. Volume was reduced *in vacuo* to about 2 ml. and again any colloidal material which appeared was removed by centrifugation. Purification of the extract with ethyl acetate and benzene was carried out as previously.

(*b*) *Paper Chromatography of the anthocyanins*

Ascending paper chromatography at 30°C. was employed in most cases. Whatman No. 1 filter paper was used generally but, for the banding of large volumes, Whatman 3-mm. was preferred. The developing solvent which gave best results was the organic phase of a butanol-acetic acid-water in the proportions of 4:1:5 by volume.

In those instances where spots were difficult to resolve, as in the case of the A and D-E groups of the purple barleys, re-chromatography was necessary. Two or three applications of the anthocyanin extract were applied to Whatman 3-mm. paper on a kymograph drum with an automatic pipette designed by the senior author. The solvent was allowed to run off the chromatogram for 3 days during which time a reasonable resolution of the anthocyanin bands was obtained. The bands were eluted with 1 per cent hydrochloric acid in methanol or ethanol and re-chromatographed on Whatman No. 1 paper to obtain Rf values.

The time required for good resolution with ascending chromatography on No. 1 paper was usually 24 hours. Some slow-moving anthocyanins found in the purple varieties tended to be unstable during solvent development and could not be assigned definite Rf values. The ferric chloride test was carefully applied to the anthocyanin spots; concentration was important and was varied to suit the circumstance from 0.05 to 1.0 per cent. Spots to be tested were cut out and slipped slowly sideways into the ferric chloride solution. Critical trials were run with cyanidin-3-glucoside alone and in combination with extracts from the eight varieties. Concentrations of the anthocyanins (and anthocyanidins) were ocularly estimated from the chromatospots. The ratios reported were always the result of the observations of two or more of the authors. Attempts at more objective presentation were not successful.

(*c*) *Hydrolysis of Anthocyanins and the Extraction of the Anthocyanidins*

The following procedure gave the best extracts for the chromatography of the anthocyanidins. To 3 grams of the powder from the purple barley pearling and 8 grams from blue, black, or white barley pearlings, 3N hydrochloric acid was added at 15 ml. per gram. Hydrolysis for 30 minutes at near boiling temperatures was followed by centrifugation at 8000 r.p.m. for 15 minutes. To the supernatant fluid, iso-amyl alcohol was added at 1 ml. per 5 ml. of extract. The passage of the anthocyanidins from water to alcohol was best achieved by adding at one time a small amount of iso-amyl alcohol, shaking vigorously, and drawing off the alcoholic phase with a pro-pipette. The procedure was repeated to leave the hydrochloric acid free of anthocyanidins. The combined alcoholic fractions to which 1 or 2 ml. of 1 per cent aqueous hydrochloric acid had been added were mixed with petroleum ether in generous amounts (five to ten times the total volume of acid-alcohol solution) with vigorous shaking. Pelargonidin particularly was difficult to move from the iso-amyl alcohol-petroleum ether to the acid-water phase. Accordingly, the procedure had to be repeated to be sure of complete displacement. After separation of the phases, 2 to 3 drops of iso-amyl alcohol were added to the acid-water phase which contained the anthocyanidins and again the anthocyanidins moved into the alcohol. Usually the iso-amyl alcohol extract was pure enough for chromatography.

(*d*) *Paper Chromatography of the Anthocyanidins*

The anthocyanidins from the barleys were chromatographed on Whatman No. 1 paper by the ascending technique, using the Forrestal

solvent (water-hydro-chloric acid-acetic acid, 10:3:30 by volume). Authentic cyanidin and pelar-gonidin were chromatographed alone and mixed with extracts as controls. To relate anthocyanins to "parent" anthrocyanidins, stripes of anthocyanin extract were applied to Whatman 3-mm. paper and bands eluted as described before. After hydrolysis of the eluates, the anthocyanidins were collected and chromatographed in the usual way.

(*e*) *Histochemical Tests for Anthocyanin in the Aleurone and Pericarp Tissues*

The caryopses of the eight barley varieties selected for the special study of anthocyanidins and anthocyanins by paper chromatography were, with others, sectioned freehand and with a sliding microtome in a manner

TABLE 1.—ANTHOCYANINS IN THE PERICARP AND ALEURONE OF EIGHT VARIETIES OF BARLEY

Variety	Plant breeders' colour classification	Characteristics	Chromato-spots; solvent front →						
			A_1	A	B	C	D	E	F
1 Golden Pheasant	White	Rf × 100	No anthocyanin present						
		colour							
		$FeCl_3$ reaction							
		concentration ratio							
2* Kwan	Blue	Rf × 100	0	0	21–22	28–30	0	0	
		colour			B-R	R			
		$FeCl_3$ reaction			B-Bl	B			
		concentration ratio			3	1			
3** Trebi	Blue	Rf × 100	0	0	21–22	28–30	0	0	
		colour			B-R	R			
		$FeCl_3$ reaction			B-Bl	B			
		concentration ratio			3	1			
4 Montcalm	Blue	Rf × 100	0	0	21–22	28–30	0	0	
		colour			B-R	R			
		$FeCl_3$ reaction			B-Bl	B			
		concentration ratio			3	1			
5 Gopal	Purple	Rf × 100	<13	13–16	21–22	28–30	36–37	41	60
		colour		B	R-Br	R	L-R	L-R	V-L-R
		$FeCl_3$ reaction			B	B		no	
		concentration ratio	< 6	> 1	2	15	3	6	> 1
6 Black Hulless	Purple	Rf × 100	<13		21–22	28–30	36–37	41	0
		colour			B-R	R	L-R	L-R	
		$FeCl_3$ reaction			B-Bl	B		no	
		concentration ratio	< 6	> 1	4	5	2	2	
7 Lion	Black	Rf × 100	No anthocyanin present						
		colour							
		$FeCl_3$ reaction							
		concentration ratio							
8 Gatami	Black	Rf × 100		0	21–22	28–30	0	0	0
		colour			R-B	R			
		$FeCl_3$ reaction			B-Bl	B			
		concentration ratio			3	1			
9 Cyanidin-3-glucoside		Rf × 100		0	0	28–30	0	0	0
		colour				R	B		
		$FeCl_3$ reaction				B			
		concentration ratio							

* More pigment than in Trebi
** Flavonoids other than anthocyanins abundant

Br Brown
R Red
B Blue
L-R Light Red
V-L-R Very light red
Bl Black

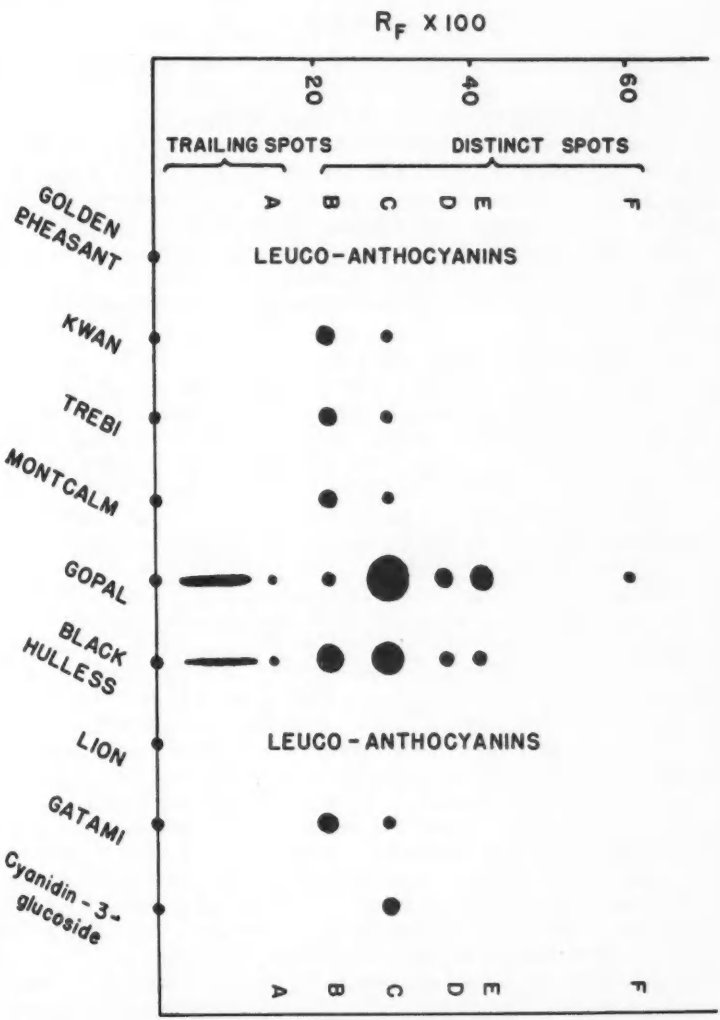

FIGURE 1. Diagrammatic representation of the anthocyanin chromatogram for eight barley varieties and authentic cyanidin-3-glucoside.

similar to that reported by Harlan (7). Sections were placed on dry slides and cover slips were fastened over them by sealing two opposite sides with paraffin. Sections were observed dry and, in the presence of 2 per cent hydrochloric acid or 2 per cent ammonium hydroxide, run under the cover slip. Some of the reactions were unsatisfactory because of the presence of coloured concomitant substances and because of variations within the caryopses of a given barley variety.

RESULTS

(*a*) *Distribution, Number, Nature, and Concentration Ratios of the Anthocyanins*

Some of the data on the anthocyanins in the aleurone and pericarp tissues of the eight selected barley varieties are given in Table I and Figure I.

Anthocyanins, it can be seen, are not present in the white barley Golden Pheasant although the presence of leucoanthocyanins can be confirmed. Two distinct anthocyanins, spots B and C, are present in the three blue varieties, Kwan, Trebi, Montcalm, and the black variety Gatami. The anthocyanin C gives the colour reactions and Rf values of cyanidin-3-glucoside. The other anthocyanin B is probably a delphinidin glycoside.

The concentration ratios, given in Table I, are to be applied only within varieties not between varieties. However, it may be noted that total pigment extracted from Montcalm was much less than from Trebi or Kwan. Trebi contained much flavonoid co-pigment which modified the anthocyanin colour *in vivo*. In all the blue varieties, there was about three times as much delphinidin glycoside as cyanidin glycoside.

In the purple varieties, Black Hulless and Gopal, anthocyanins corresponding to spots B, C, D, and E are distinct. Anthocyanins B and C in Black Hulless are chromatographically the same as those in the blue varieties. In the variety Gopal, anthocyanin C is chromatographically identical with anthocyanin C of Black Hulless and of the blue varieties, but anthocyanin B may differ in the glycosidic part of the molecule. It is rusty red rather than bluish red in colour. The concentration ratio of the B and C anthocyanins in the two purple varieties is very different. In both purple varieties, there occurred a mixture of red-brown compounds designated in Table I as A_1 and A which could not be readily resolved in the chromatograms. The components of the group did not separate on elution and re-chromatography, and they oxidized rapidly to blue-black materials. Delphinidin derivatives occur in the mixture but whether only these are present is uncertain. Anthocyanins D and E were found only in the purple varieties. Anthocyanin E is a pelargonidin derivative and its colour is not altered by ferric chloride. Distinctive ferric chloride reactions could not be obtained with D. The spot F was always given by extracts from Gopal but not from Black Hulless; it was perhaps an anthocyanin, but too little was present to give a reliable ferric chloride reaction. In Gopal the major anthocyanins contributing to colour were C, E, and D; in Black Hulless, B and C. Gopal yielded more pigment than Black Hulless but both gave more than any of the blue or black varieties.

Lion, a black variety, yielded no anthocyanin but Gatami, the other black variety, gave two anthocyanins, B and C, which appeared to be identical to and in about the same concentration ratio as the B and C anthocyanins of the three blue varieties.

(*b*) *Distribution, Number, and Concentration Ratio of Anthocyanidins*

The data for the anthocyanidins of the aleurone and pericarp tissues of the eight barley varieties are given in Table 2 and Figure 2. Cyanidin

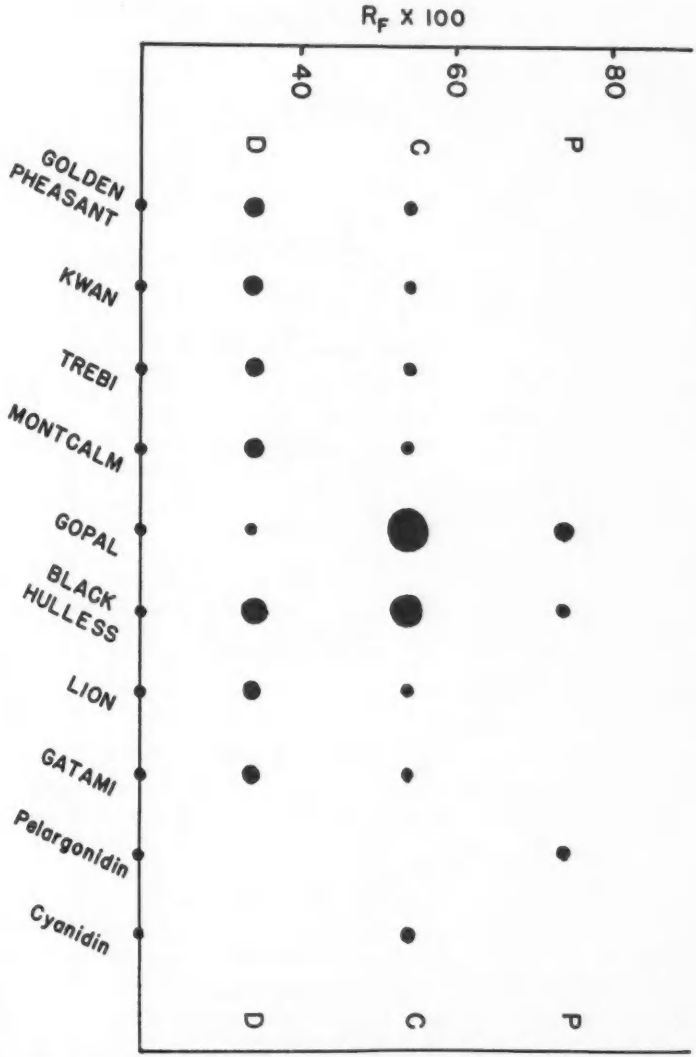

FIGURE 2. Diagrammatic representation of the anthocyanidin chromatogram for eight barley varieties and authentic pelargonidin and cyanidin.

and delphinidin were obtained from the caryopses of all varieties and, in all but the purple varieties, the delphinidin was more plentiful than the cyanidin. In the purple varieties, cyanidin occurred in relatively larger amounts than delphinidin and, in addition, pelargonidin was present.

TABLE 2.—ANTHOCYANIDINS IN THE PERICARP AND ALEURONE TISSUES OF EIGHT VARIETIES OF BARLEY

Variety	Breeders' colour class	Characteristics	Chromato-spots; solvent front→		
			D	C	P
1		Rf × 100	34	54	
Golden Pheasant	White	colour	B-R	R	
		$FeCl_3$ reaction	B	B	
		concentration ratio	3	1	
		U.V.	B-R	R	
2		Rf × 100	34	54	
Kwan	Blue	colour	B-R	R	
		$FeCl_3$ reaction	B	B	
		concentration ratio	3	1	
		U.V.	B-R	R	
3		Rf × 100	34	54	
Trebi	Blue	colour	B-R	R	
		$FeCl_3$ reaction	B	B	
		concentration ratio	3	1	
		U.V.	B-R	R	
4		Rf × 100	34	54	
Montcalm	Blue	colour	B-R	R	
		$FeCl_3$ reaction	B	B	
		concentration ratio	3	1	
		U.V.	B-R	R	
5		Rf × 100	34	54	74
Gopal	Purple	colour	B-R	R	OR
		$FeCl_3$ reaction	B	B	OR
		concentration ratio	1	15	5
6		U.V.	B-R	R	O-R
Black Hulless	Purple	Rf × 100	34	54	74
		colour	B-R	R	OR
		$FeCl_3$ reaction	B	B	OR
		concentration ratio	5	15	1
		U.V.	B-R	R	O-R
7		Rf × 100	34	54	
Lion	Black	colour	B-R	R	
		$FeCl_3$ reaction	B	B	
		concentration ratio	3	1	
		U.V.	B-R	R	
8		Rf × 100	34	54	
Gatami	Black	colour	B-R	R	
		$FeCl_3$ reaction	B	B	
		concentration ratio	3	1	
		U.V.	B-R	R	
		Rf × 100			74
Pelargonidin		colour			OR
		$FeCl_3$ reaction			OR
		U.V.			OR
Cyanidin		Rf × 100		54	
		colour		R	
		$FeCl_3$ reaction		B	
		U.V.		R	

B-R Bluish red
R Dark red
B Blue
OR Orange red

TABLE 3.—HISTOCHEMICAL OBSERVATIONS FOR ANTHOCYANINS

Variety	Dry Mount		2% HCL		2% NH_4OH	
	Aleurone	Pericarp	Aleurone	Pericarp	Aleurone	Pericarp
White Golden Pheasant	− C	− C	− C	− C	− Y-O	− Y
Blue Kwan	+ B	B-Bl	+ R	O-Y	+ B	Br-O
Trebi	+ B	Br	+ R	O-Y	+ B	O-Y
Montcalm	+ B	− O	+ R	− O	+ Y-G	− O
Purple Gopal	+ B	+ R-B	+ R	+ R-B	+ G	+ G-B
Black Hulless	− C	+ R	+ R	+ R	+ G	+ O-G
Black Gatami	+ B	− Bl	+ R-B	− Bl	+ Y-G	− Bl
Lion	− C	− Bl	− C	− Bl	− Y	− Bl

\+ Anthocyanin probably present
− Anthocyanin not likely present or masked

C Colourless
Y Yellow
O Orange
B Blue
R Red or pink
G Green
Bl Black
Br Brown

No differences in the behaviour of the barley anthocyanidins and the available authentic anthocyanidins were observed when they were studied singly, in mixtures, in the ferric chloride test, or under short-wave ultraviolet fluorescence.

The relationships of specific anthocyanins to possible "parent" anthocyanidins were well established by hydrolysis of eluted anthocyanins and rechromatography. Thus anthocyanin C is, beyond doubt, a cyanidin derivative; B, a delphinidin derivative, and E, a pelargonidin derivative. D, which may be a cyanidin or pelargonidin derivative, gave weak reactions which could not be called distinctive. The A_1-A group of anthocyanins found in the purple varieties gave delphinidin reactions but the members could not be well separated because of their apparent instability.

(*c*) *The Histochemical Tests*

Many compounds other than anthocyanins appear to contribute to some extent to the colour of barley aleurone and pericarp tissues. It is, therefore, not surprising that the histochemical tests were somewhat unsatisfactory. Some results nonetheless are given (Table 3) for, in some instances, they confirm and extend other observations. For example,

histological differences in black varieties confirm the observations of extraction and chromatography, viz., Lion from which anthocyanins were not extracted had a colourless aleurone and a melanin-black pericarp, while Gatami which gave two anthocyanins had a coloured aleurone and a black pericarp. Purple varieties showed colour in both pericarp and aleurone, but increased colour in the pericarp was accompanied by decreased colour in the aleurone and vice versa. In blue varieties, most of the pigment occurred in the aleurone tissue and gave the typical acid-base reaction of anthocyanins. Orange or yellow flavonoid pigments occurring in the pericarp did not give anthocyanin reactions. They tended to mask blue anthocyanins and, in their presence, to give a green tissue colour in alkali. White varieties showed orange or pink colours in aleurone and pericarp and did not give good anthocyanin reactions.

DISCUSSION

Difficulties are encountered in the study of the anthocyanins of the barley caryopsis which are not met in other parts of the plant. In the first place, the aleurone and pericarp tissues, which contain anthocyanins, differ genetically and cannot be separated from one another or from the chaff and endosperm. At best the tissues can be concentrated as dust from pearling the whole barley grain. Pigment develops late in the caryopsis and appears to be singularly subject to influence by changing environmental conditions. Accompanying the anthocyanins in aleurone and pericarp are other related flavonoid substances and large amounts of reserve protein and carbohydrate. Before the anthocyanins can be resolved chromatographically, concentrated extracts of reasonable purity must be obtained. The amount of extract spotted on chromatogram loci must be determined within very narrow limits to prevent overloading and trailing on the one hand or weak development on the other. This difficulty may be associated with the variation in glycosidation of the parent anthocyanidins which in turn may reflect the physiological state of the plant on a given day or week.

The anthocyanin chemistry as so far developed can be related to some extent to the colour genetics of the caryopsis. Myler and Stanford (13) and later Briggs and Stanford (3) stated that blue barley colour was importantly concerned with two complementary, independently assorting gene pairs Bl and Bl_2. Thus the genotype of pure breeding Kwan, a barley with blue aleurone, would be designated as Bl Bl, Bl_2 Bl_2; Goldfoil, a white barley, as bl bl, Bl_2 Bl_2; and Napal, another white barley, as Bl Bl, bl_2 bl_2. Leucoanthocyanin, in the only white barley studied, yielded two anthocyanidins, delphinidin and cyanidin, in the approximate concentration ratio of 3:1. In the three blue varieties and in one of the black varieties examined, two anthocyanins were found: one a delphinidin, and one a cyanidin derivative. Superficially it would appear that two gene-controlled steps are required to develop the two anthocyanins from two leucoanthocyanins. Robinson and Robinson (14) reported leucoanthocyanins in barley. Bate-Smith (2) noted that almost all leucoanthocyanins yield cyanidin and/or delphinidin; Harris (9) and McFarlane, Wye, and

Grant (12) found cyanidin, delphinidin, and several unidentified anthocyanidins in barley malt. To this point, then, investigations form a consistent pattern.

Very little can be said of the relationships of the chemistry and the genetics of the purple factors. Harlan (8) believed that purple caryopsis was attributable to a red pericarp underlain by a blue aleurone but this statement, although generally true, may prove to be too simple. Until the work of Woodward and Thieret (18), most barley specialists believed "purple seed" to be simply dominant over "colourless seed". However, they were able to show that two independently assorting, complementary gene pairs, P_p and C_c, were involved. Our studies would support the involvement of at least two gene pairs in the two purple varieties Gopal and Black Hulless. The inheritance pattern, however, is not a simple imposition of "the pattern for purple" on "the pattern for blue". In the purple grains, perlargonidin derivatives, which do not appear in the blue grains, occur and the delphinidin and cyanidin derivatives, which appear in the blue grains, are in very different concentration ratios. Moreover, there is a decided difference between the two purple varieties in the distribution and number of their anthocyanins. It might be reasonable to assume that determination involves precursors of the anthocyanidins.

Harlan (7), as mentioned earlier, noted the melanin-like pigment in the pericarp of the black barleys. Buckley (4), studying the inheritance of "black" and "white", reported monofactorial patterns. It seems probable that the chemistry of melanin pigments is quite unrelated to that of the anthocyanins. Nevertheless it would be of interest to know if a full range of anthocyanins is found in the black varieties. Our variety, Gatami, was "blue" masked by "black"; perhaps other varieties are "purple" masked by "black".

ACKNOWLEDGEMENTS

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SOME BIOCHEMICAL CHANGES ON STORAGE IN POTATOES FROM PRINCE EDWARD ISLAND, AND THEIR RELATION TO THE QUALITY OF CHIPS[1]

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ABSTRACT

Different varieties of potatoes were characterized by differences in their content of water, starch, and reducing sugar, and in susceptibility to change in response to the surrounding temperature.

A critical concentration of 0.4 per cent reducing sugar was found, above which chips were dark brown, and unacceptable in flavour. Storage at 35° F. caused a rise in the concentration of reducing sugar in all varieties to between 1.5 and 4.0 per cent in 5 to 14 weeks. During storage at 50° F. the reducing sugar usually rose above 0.4 per cent in 9 to 13 weeks.

Shrinkage and rot developed markedly in the Sebago, and to a less extent in the Katahdin, when the temperature was raised from 50° or 35° to 70° F., and the chips produced from them were poor in texture and flavour.

A fall in the level of reducing sugar below 0.4 per cent occurred in 3 to 9 weeks during conditioning at 70° F. in the Netted Gem, Green Mountain, and Irish Cobbler varieties, and the chips from these were acceptable.

The biochemical behaviour of the Netted Gem and Irish Cobbler was most conducive to the production of acceptable chips.

INTRODUCTION

A knowledge of the biochemical characteristics of potatoes which are conducive to the production of acceptable chips is of commercial importance in Canada (1). Factors affecting these characteristics are mainly the variety of potato, the conditions of growth, and the treatment in storage after harvest.

In 1882 Müller-Thurgau (7) discovered that reducing sugar accumulated in potato tubers when they were exposed to low temperature. The degree of accumulation depended upon the degree of lowering of the temperature, and the process could be reversed by raising the temperature.

Potatoes differ according to variety in the rate at which reducing sugar accumulates in cool storage (3, 10, 11, 12, 15), and the rate at which it disappears when the temperature is raised (15). Such differences may also be imposed by conditions of growth (11, 17).

In 1936 Wright *et al.* (16) reported that, as the reducing sugar content of potatoes increased, chips made from them became darker in colour and the flavour was adversely affected. Results of further work made it clear that the amount of reducing sugar in the potato is one of the most important factors in the production of acceptable chips (2, 10). A level of 0.4 per cent has been found to be the upper limit conducive to a satisfactory product (2, 6, 17).

A high specific gravity, which corresponds to a high content of total solids and starch, is also associated with the production of chips of good quality (6).

[1] Issued as N. R. C. No. 4933.

There is some information on the biochemical properties associated with the production of good chips from potatoes grown in Canada (1, 5, 14). The present investigation was undertaken to supply information on several varieties grown in Prince Edward Island. It involved a study of the effect of storage at different temperatures on certain chemical characteristics, and the relationship of these factors to the production of acceptable chips.

MATERIALS AND METHODS

The potatoes were grown at the Experimental Station in Charlottetown, Prince Edward Island, in 1954 and 1956. Irish Cobbler and Sebago from each year's crop were included in the study. Green Mountain and Katahdin were the other varieties in 1954, and Kennebec and Netted Gem in 1956.

Potatoes of the 1954 crop were grown in a 3-year rotation of potatoes, grain, and clover. Details of fertilization are given in Figure 1. Those of the 1956 crop were grown on fall-ploughed sod land, with 6-12-12 fertilizer applied at 1800 lb. per acre.

In each year the potatoes were harvested in the latter part of October, stored at 40° to 55° F. for 3 to 4 weeks, then shipped to Halifax.

In the laboratory, portions of each lot were stored at 70°, 50°, and 35° F. Those stored at the two lower temperatures were eventually moved into the room at 70° F. for study of the conditioning process. Some of each lot were kept at 75° F. for the first analyses and tests.

One of the lots of Sebago in 1956 was treated with sprout inhibitor* at the laboratory before storage. The material was mixed with water and applied at a level of 0.5 g. per bushel.

At various times the levels of total solids, starch, protein, and reducing sugar were determined on the potatoes, and the quality of chips made from them was tested. For analyses and tests they were always peeled, and samples of 8 to 10 tubers, including a variety of sizes, were used.

Total solids were estimated on samples of brei made from equal weights of potato tissue and water mixed in a Waring blendor. The water was evaporated at 80° C. in an oven with a forced draft. The dish and contents were cooled in a desiccator before the final weighing. Starch was estimated by the method of Nielsen and Gleason (8, 9), and reducing sugar was measured colorimetrically with sodium-2,4-dinitrophenolate (11). Total nitrogen was determined by a titrimetric micro-Kjeldahl technique (4, 13), the factor 6.25 being used for conversion to protein.

High grade cottonseed oil was used as the cooking fat for chips. Slices were cut 1/16 in. thick in a food-slicing machine, rinsed in cold running water to remove surface starch, then dried between paper towels. The oil was heated to 385° - 390° F. before the slices were put in. They were cooked until crisp. This usually required $1\frac{3}{4}$ minutes and coincided with the cessation of bubbling. The chips were drained on paper towels, and lightly salted before being assessed for colour, flavour, and texture.

* Chloro I P C. (Niagara Brand Spray Co., Burlington, Ont.).

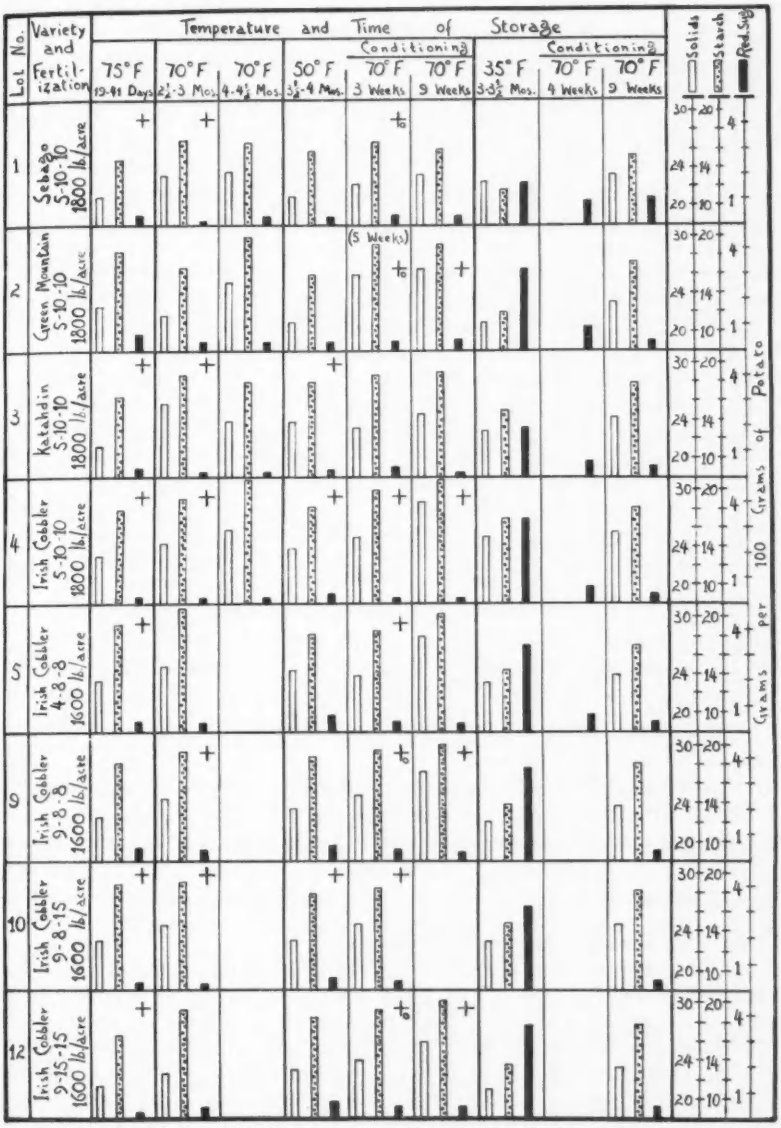
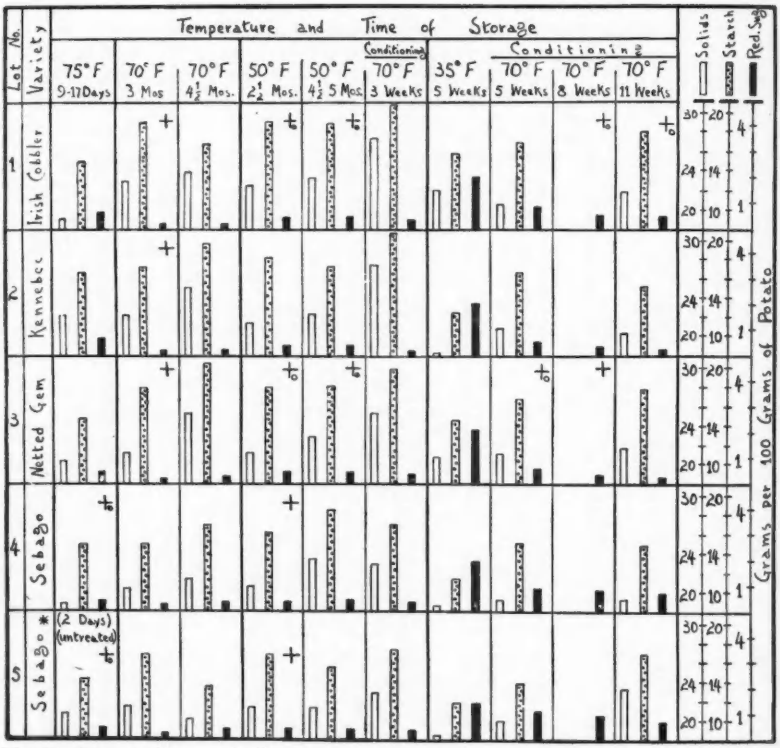


FIGURE 1. Biochemical changes and quality of chips during storage in potatoes of the 1954 crop.

RESULTS AND DISCUSSION

Figures 1 and 2 illustrate the changes which occurred during storage at the various temperatures. Values for protein are not shown because they varied little, and there was no suggestion that they were related to



*Lot No. 5 treated with sprout inhibitor

FIGURE 2. Biochemical changes and quality of chips during storage in potatoes of the 1956 crop.

the quality of chips. The symbol + indicates that the chips resembled the commercial product, and the symbol $+_o$ that they were darker and more highly flavoured. When no symbol is used the chips were unacceptable.

Differences in Initial Chemical Composition

Compared to other varieties, the Sebago from both years showed a low content of total solids. In 1954 it was the lowest in starch, and in 1956 the starch content was about average. The highest values for total solids and starch were shown by the various lots of Irish Cobbler in 1954.

The initial levels of reducing sugar in all the potatoes were generally high, but the studies in 1954 showed that they decreased considerably over a relatively short time when the tubers were kept at the temperature of the laboratory. The high values were probably the result of cool storage between the time of harvesting and shipping. The comparatively low level of reducing sugar in the Sebago of both years is particularly noteworthy, and this was probably the most important factor which allowed the preparation of acceptable chips from this variety of potato in the initial stages of the experiment.

Differences in Response to Storage and Conditioning

In both years, but particularly in 1954, in the Irish Cobbler there was a small rise in reducing sugar during cool storage, and it fell below the 0.4 per cent level in a relatively short time at conditioning temperature. Green Mountain and Netted Gem were the only other varieties which showed this property in comparable degree.

Another important factor affecting the quality of chips was associated with withering. When the tubers became very withered, and at the same time in most cases sprouted, the chips made from them were somewhat flabby, and stale in taste. This was particularly evident in certain lots when they were kept at room temperature or conditioning temperature. It was important in the case of the Sebago and Katahdin varieties, which were characteristically high in water.

The Sebago of both years showed a low content of solids and starch. It was always the first to sprout, and it withered faster than any other variety. Its initial content of reducing sugar was not as high as that of most varieties, and it did not increase greatly during storage at 50° F. At conditioning temperature after cool storage, however, the level of reducing sugar did not fall, and at the same time there was rapid general deterioration of the potato. Storage at 35° F. had an even more adverse effect on the Sebago.

Differences Due to Seasonal Conditions

Levels of total solids and of starch were higher in potatoes of the 1954 crop than in those of the 1956 crop. These differences probably were not altogether associated with variety, because they were evident in the Sebago and the Cobbler, the two varieties which were examined from each year's crop.

There was a small difference in rainfall at the Experimental Station in Charlottetown during the growing season in those 2 years. In the 7 consecutive months, including April and October, there were 18.8 inches in 1954 and 19.2 inches in 1956. This difference was mainly because there was considerably more rain in the first 3 months of the period in 1956. There was considerably less in both July and August of that year, the same in September, and considerably less in October*.

Differences in Fertilization, and the Use of Sprout Inhibitor

From our data it was not possible to distinguish biochemical differences which might be related to differences in fertilization.

The sprout inhibitor was effective, and it also retarded the process of withering; but it was not associated with any important differences in fluctuations of the levels of water and carbohydrate under the various conditions of storage. The treated potatoes, like the untreated lot of Sebago, showed the same fast degenerative changes when they were taken from cool storage.

*Information on rainfall supplied by the Dominion Public Weather Office in Halifax, N.S.

Differences in Quality of Chips as Related to Chemical Composition

The level of reducing sugar in the tubers was the most important chemical factor associated with the quality of chips. It was practically impossible to make acceptable chips if the concentration of reducing sugar was above 0.4 per cent. This is in agreement with the work of others on potatoes grown in the United States. Potatoes containing 0.3 to 0.4 per cent generally made chips darker in colour and stronger in flavour than those which are sold commercially. Potatoes containing up to 0.3 per cent yielded chips of a light cream colour and mild flavour, in both respects resembling the usual commercial product. If the period of storage had been short, good chips could be made from any of the potatoes if the level of reducing sugar was satisfactory. At this stage other chemical properties did not seem to be important to the production of acceptable chips.

The potato with the highest levels of solids and starch initially was the Cobbler grown in 1954 with 4-8-8 fertilizer. Although under some conditions good chips could be made from it, the level of reducing sugar was troublesome, and behaved in such a way that this lot would have presented too much risk for use in the commercial production of chips. The Cobbler from the 1956 crop had a lower content of solids and starch than any potato from the 1954 crop, and its level of reducing sugar did not behave so well in conditioning. The tubers remained firm, however, for a longer time after sprouting began than did most of the potatoes from the 1956 crop.

The Netted Gem (1956 crop) was slower in sprouting than any of the other varieties, and retained firmness better. The level of reducing sugar responded well to conditioning temperature. Thus in this potato there was a combination of two important factors associated with the production of good chips. It produced good chips not only when it was kept for a few months at 70° F., but it was the only one which could be satisfactorily conditioned after storage at 35°F. In practice, of course, this treatment would not be used; it is a rigid test of conditioning ability. The possibilities of this variety of potato as a source of chips were the best of any.

ACKNOWLEDGEMENT

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A NEW ROOT ROT OF FLORISTS' CHRYSANTHEMUMS IN ONTARIO[1]

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ABSTRACT

An apparently undescribed root rot of *Chrysanthemum morifolium* Ramat. occurred in 1953 on the horticultural variety "White Shasta" in an Ontario greenhouse. The disease was characterized by a severe root rot, general stunting and a foliar chlorosis and necrosis. A species of the form genus *Phoma* was found associated with the roots of affected plants. The disease has been reproduced repeatedly by inoculation with monoconidal cultures of the fungus. Symptoms appear to be most severe on plants growing in infested soil maintained at temperatures between 55° and 60°F. Inoculation tests suggest that the disease is restricted to the florists' chrysanthemum. Varieties of *C. morifolium* do, however, vary widely in their susceptibility to this root rot. There is no evidence that the pathogen is carried within cuttings taken from infected plants. Both steam sterlization and methyl bromide fumigation have completely eliminated the pathogen from infested soils.

INTRODUCTION

In June 1954, the author published a preliminary report on a hitherto unrecognized root rot of the florists' chrysanthemum variety "White Shasta", caused by an undetermined species of the genus *Phoma* (1). Since that time, this disease or one producing a very similar syndrome on "White Shasta" has been reported to have occurred at scattered points throughout southern Ontario. Furthermore, chrysanthemums with badly rotted roots, and suspected by the grower of being affected by Phoma root rot, have been submitted periodically to the St. Catharines Laboratory for examination. However, only two authentic cases of this disease have been encountered during the past 3 years and both cases were found in widely separated greenhouses, in the vicinity of Weston, Ontario, the scene of the first outbreak.

Although the disease appears at present to be restricted to one particular area in Ontario, the severity of the original attack was sufficient to establish this root rot as a potentially serious problem for chrysanthemum growers and to emphasize the necessity for a more detailed study of various phases of disease development and methods of control. Accordingly, a series of experiments was carried out and the results are presented in this paper.

LITERATURE REVIEW

A review of the literature failed to reveal any record of a species of *Phoma* that had been considered responsible for a root rot of *Chrysanthemum* spp. in North America.

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However, in 1953, Srivastava (2) working at the Plant Quarantine Station, Poona, India, intercepted several varieties of chrysanthemum species that exhibited a root rot with which a species of *Phoma* was associated. These varieties came from Holland. The fungus closely resembled *Phoma chrysanthemicola* Hollos in its morphological characters and consequently it was referred to this species. Srivastava was of the opinion that this organism had been carried on these plants from Holland to India, although unreported previously from either of these countries.

SYMPTOMS OF THE DISEASE

The primary symptoms are restricted to the roots which exhibit reddish brown lesions of an indefinite size and margin. As the disease advances, the tips of many of the finer secondary roots of the adventitious root system are killed. In severely affected plants of susceptible varieties such as "White Shasta", only a few fragments of discoloured root remain attached to the base of the stem. New roots, if or when they are produced, are progressively infected and become functionless (Figure 1, A). The loss of many of the feeding rootlets which constitute a large part of the root system induces extreme dwarfing (Figure 1, C). It is remarkable that, although the roots are frequently badly rotted, no permanent wilt of stunted plants occurs.

As an indirect result of the root injury, the foliage of infected plants becomes slightly chlorotic, followed in many instances by the development of brown, necrotic areas which coalesce, and by ultimate leaf desiccation (Figure 1, B). The affected leaves remain attached to the stem and finally shrivel along its length. The foliage symptoms begin to appear on infected plants 7 to 14 days after benching.

Actually, these symptoms alone are unreliable, as many of the changes which appear on *Phoma*-infected plants closely resemble those incited by a nutrient deficiency, root knot nematode, certain soil insects and excessive salinity (3, 4).

Observation of free-hand longitudinal sections of slightly infected root material, stained with cotton blue in lactophenol, suggests that the fungus is mainly a cortical pathogen.

GENERAL METHODS

Repeated isolations were made during March 1953 from the leaves, stems and roots of numerous "White Shasta" plants which exhibited various degrees of stunting. The tissue from chlorotic and necrotic areas on the foliage of affected "White Shasta" plants failed to yield fungi or bacteria. Various bacteria but no fungi were isolated from the stem tissue. Many fungi, however, were found associated with the rotting roots. Of these, isolates of a species of *Phoma*, all of which appeared to be morphologically similar on potato-dextrose agar, occurred most consistently. Later, the same fungus was recovered from reddish brown lesions found on the roots of ten additional varieties of *C. morifolium* which had been grown in naturally infested soil. Again, in January 1955, specimens of "White Shasta" from two different locations yielded a species of *Phoma*.

There also appeared in culture members of the genera *Penicillium*, *Aspergillus*, *Fusarium*, *Alternaria*, and numerous bacteria.

In the early experiments, inoculum for pathogenicity studies was prepared by growing the fungus isolates on cornmeal-sand medium. Later this substrate was replaced by a sugar-beet pulp medium.

The inoculum was thoroughly incorporated with freshly sterilized soils. The amount of inoculum added to the soil varied from experiment to experiment, but in any one test it was maintained at the same level. Controls were treated in the same way, except that sterile sugar-beet pulp only was mixed with the soil. Because of their extreme susceptibility to this root rot, the florists' chrysanthemum varieties "White Shasta", "Yellow Seagull", and "Pink Mistletoe" were used exclusively in the pathogenicity studies. Only newly rooted cuttings of the test varieties were employed in the various phases of the investigation.

The effect of temperature and H-ion concentration on the growth of the pathogen was determined by measuring and then by averaging the increase in diameter of the colonies in a replicated series of Petri dishes containing 10 ml. of potato-dextrose agar. Incubators in which the temperature ranged from 5 to 30°C., varying by 5° intervals, were used in the study of this factor. Various pH levels from 4.0 to 8.0 were established by adjusting autoclaved potato-dextrose agar just before solidification with sterilized hydrochloric acid and sodium hydroxide.

Generally, the analysis of variance has been used to calculate a generalized standard error for comparing differences between specific treatments in the same test. On occasion, in the pathogenicity tests, in order to determine whether mean differences in height and fresh weight were mathematically significant, the data were analysed by Student's 't' method.

ETIOLOGY

Pathogenicity

Two preliminary tests, in which the varieties "White Shasta" and "Yellow Seagull" were grown in naturally infested soil, and in the same soil either leached or steamed, suggested that the root injury was of biotic rather than of abiotic origin. Soil analyses made prior to the leaching also failed to give any indication that the condition was a result of a nutritional disorder.

Of the several fungi associated with this root necrosis, only the *Phoma* and *Fusarium* species were tested for pathogenicity. In less than 3 weeks, root rot symptoms were apparent on all the plants grown in the soils artificially infested with the *Phoma* sp. and with the *Phoma* and *Fusarium* spp. together. Inasmuch as the *Fusarium* sp. alone failed to initiate the disease whereas the *Phoma* sp. did, it seemed logical to consider the *Phoma* sp. as the primary causal agent.

Subsequently six series of inoculations involving 160 plants of the varieties "White Shasta" and "Pink Mistletoe", were carried out from August to December 1953, to establish the pathogenicity of ten monoconidial isolates which culturally appeared morphologically similar. Again, in 1955, isolate WD1, which had been maintained on potato-dextrose agar

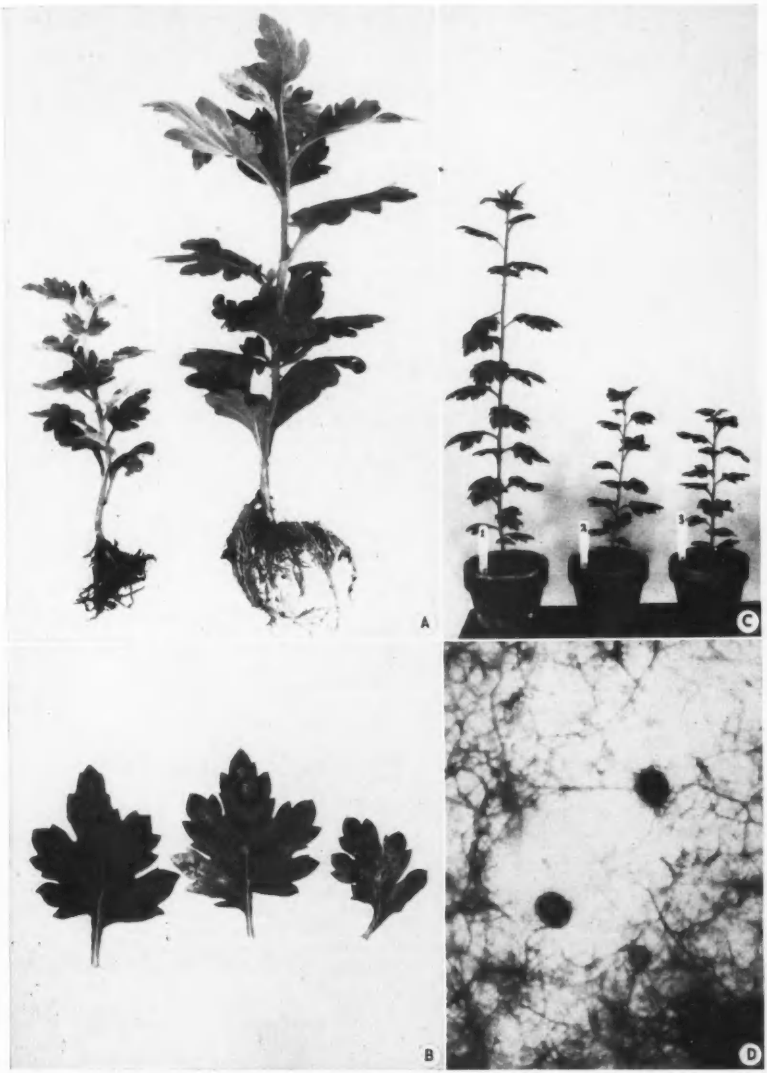

FIGURE 1, A. Effect of *Phoma* sp. WD1 on the roots of the chrysanthemum variety White Shasta; diseased plant (*left*) grown in artificially infested soil; healthy plant (*right*) grown in sterilized soil.

B. Leaves from White Shasta showing the indirect effects of the pathogen.

C. The effect of the pathogen on the size of White Shasta.

D. Pycnidia of *Phoma* sp. WD1 produced on potato dextrose agar. (approx. x40).

TABLE 1.—SUMMARY OF RESULTS OF GREENHOUSE PATHOGENICITY TESTS

Expt. no.	Variety	Soil treatment	Duration of expt. weeks	No. plants		Mean fresh weight, gm.
				inoculated	infected	
1	White Shasta	Naturally infested, untreated	12	15	15	5.9
		Naturally infested, steamed		6	0	31.5
2	Yellow Seagull	Naturally infested, leached	8	7	7	2.9
		Naturally infested, unleached		7	7	2.5
		Naturally infested, steamed		4	0	20.2
3	White Shasta	Phoma WD1 infested	8	5	5	—
		Fusarium sp. infested		5	0	—
		P.+F. infested		5	5	—
4	White Shasta	Phoma WD1 infested	8	5	5	14.5
		non-infested		5	0	33.0
	Pink Mistletoe	Phoma WD1 infested		4	4	16.0
		non-infested		3	0	63.1
5	White Shasta	Phoma WD1 infested	11	10	10	14.9
		Phoma WD2 infested		5	5	19.0
		non-infested		6	0	45.9
6	White Shasta	Phoma WD1 infested	6	5	5	—
		Phoma WD8 infested		5	5	—
		Phoma WD10 infested		5	5	—
		non-infested		5	0	—
	Pink Mistletoe	Phoma WD9 infested		5	5	—
		Phoma WD2 infested		5	5	—
		Phoma WD7 infested		5	5	—
		Phoma WD4 infested		5	5	—
		non-infested		5	0	—
7	Pink Mistletoe	Phoma WD1 infested	14	14	14	19.6
		Phoma WD3 infested		5	5	28.5
		Phoma WD5 infested		5	5	17.1
		Phoma WD6 infested		5	5	24.3
		non-infested		7	0	45.1
8	Yellow Shasta	Phoma WD1 infested	12	25	25	15.4
		non-infested		25	0	25.1
9	White Shasta	Phoma WD1 infested	8	30	30	11.5
		non-infested		10	0	26.9
10	White Shasta	Phoma WD1 infested*	4	7	7	—
		non-infested		4	0	—
11	White Shasta	Phoma WR2 infested	8	10	10	5.3
		non-infested		10	0	13.8

*Phoma WD1 cultured for 2 years

for 2 years, was retested to ascertain whether it was still pathogenic. Finally, isolate WR2, which was obtained from the roots of "White Shasta" in 1955 at a different greenhouse location, was tested for its pathogenicity. The results of these various experiments are listed in Table 1.

Each of the *Phoma* isolates employed in the soil inoculation experiments produced characteristically diseased plants. The pathogen was readily reisolated from infected root tissues, although much more often in some cases than in others. The frequency of recovery of the *Phoma* sp. seemed to depend to a great extent on the condition of the affected roots at the time of isolation. The identity of these re-isolates was established by a comparison with the stock cultures of the isolates. The pathogenicity of the re-isolates (WD1 and WR2) has been demonstrated.

In some series the mean fresh weight of the plants devoid of their root systems has been included to compare the growth of a particular variety in infested and non-infested soils under the same conditions. In Experiment 8, Table 1, the mean fresh weight of 25 healthy check plants, grown for a 3-month period, exceeded the mean of the same number of infected plants by 9.7 gm. The results of Experiment 9 followed the same pattern. Here, the mean fresh weight of the healthy plants grown only for 2 months exceeded by 15.4 gm. those of the diseased plants grown under the same conditions. The mean difference between the fresh weight of healthy plants and of diseased plants in these experiments was shown to be significant. These differences obviously can be attributed to the effect of the pathogen. Although there are differences in mean fresh weight of plants in these series of inoculation experiments with the monoconidial isolates of *Phoma* which may be mathematically significant, it is not suggested that these differences represent variations in virulence among the isolates. Frequently, the inoculum potential varied within a single experiment. Furthermore, the environmental conditions and the duration of the test period from one experiment to another was never constant. Finally, Phoma WD1 maintained its ability to produce the root rot even after a 2-year period in culture (Experiment 10).

Usually species of *Phoma* are associated with diseases of the stem and sometimes the foliage of the host. Consequently, the above-ground portion of ten young, healthy "White Shasta" plants was atomized with conidial suspensions of isolate WD1 on two separate occasions. A corresponding number of plants, sprayed with sterile distilled water, were maintained as controls. The inoculated plants were kept for 72 hours in a moist chamber at 60°F., the optimum temperature for the development of most florists' chrysanthemum varieties. Disease symptoms were not produced on any of the plants after 1 month.

In 1954, seedlings of the following species or horticultural varieties of *Chrysanthemum* were grown in naturally infested soil and in naturally infested soil steam sterilized prior to planting: *C. coronarium* L., *C. parthenium* Pers. var. "Golden Snowflake", *C. coccineum* Willd. var *Wilsonii*, *C. leucanthemum* L., *C. ircutianum* Turz, *C. maximum* Ram., *C. parthenium* Pers. var *pleorum*, *C. tangetum* Karsch., *C. viscido-hirtum* Thell, *C. boreale* Fedtsch., *C. oreades* (Boiss.) Wehrh., *C. segetum* L., *C. caucasicum* (Willd.) Pers., *C. corymbosum* L., and *C. balsamita* L. None of these species was susceptible to the *Phoma* root rot organism.

In an attempt to establish the identity of the chrysanthemum pathogen and to determine whether it was related to any of the *Phoma* species known to attack vegetable crops, cabbage, cauliflower, kale, kohlrabi, beet, celery

and onion were grown in WD1-infested soil. The results furnished no evidence that the chrysanthemum pathogen should be identified with any of the *Phoma* species that cause root rot of these vegetables.

The Pathogen

The causal agent was identified as a member of the form genus *Phoma*. Specific identification was attempted only in the case of *Phoma* WD1 which had been demonstrated repeatedly to be responsible for the chrysanthemum root rot under investigation. This isolate was studied in pure culture and its morphological characteristics were compared with those of definitely identified *Phoma* species recorded in the literature as associated with a disease of *Chrysanthemum* spp.

The mycelium of Phoma WD1 is septate, branched, hyaline when young, becoming brownish with age. Pycnidia are at first light brown, later black, mostly sub-globose, membranous, with a round, often indistinct, beaked ostiole, and measure 96-240 μ x 95-160 μ (Figure 1, D). The conidia are hyaline, continuous, usually oblong, sometimes botuliform, biguttulate and measure 3.2–6.4 μ x 1.6–2.4 μ. They are exuded from the pycnidum in a gelatinous matrix and are released therefrom by water. Conidia have been observed to germinate on water agar in 24 hours. Pseudosclerotial masses, dark brown to black, irregular in shape and size, are present in abundance in culture. These structures may or may not represent an early stage in the formation of the pycnidia. As yet, they have failed to develop into mature pycnidia. Habitat is living roots of *Chrysanthemum morifolium* var. "White Shasta", Weston, Ontario.

Phoma WD 1 has certain characteristics which resemble those described in the literature for *Phoma chrysanthemicola* Hollos. Unfortunately it was not possible to obtain a culture of *P. chrysanthemicola* from Srivastava in India. However, he kindly sent chrysanthemum stems, which had been preserved in formal-acet-alcohol and on which pycnidia were found.

The pycnidia and conidia of the two organisms were similar in size, shape and colour. However, pseudosclerotial masses which were common in cultures of *Phoma* WD1 were not found on the infected plant material

TABLE 2.—EFFECT OF EXCESSIVE WATERING ON DISEASE INCIDENCE AND GROWTH OF FLORISTS' CHRYSANTHEMUMS AT VARYING SOIL TEMPERATURES

Soil temperature, °F.	Root rot incidence			Growth (mean fresh weight, gm.)		
	Soil treatments			Soil treatments		
	non-infested dry	infested dry	infested wet	non-infested dry	infested dry	infested wet
50	0/4*	4/4	4/4	22.4	2.7	1.9
55	0/4	4/4	4/4	28.5	2.6	2.1
60	0/4	4/4	4/4	31.1	3.1	1.6
65	0/4	4/4	4/4	17.2	3.7	1.2
70	0/4	4/4	4/4	16.5	4.1	1.7

*Numerator = no. plants infected
Denominator = no. plants inoculated

LSD (5%) = 3.4 gm. between any
LSD (1%) = 4.6 gm. 2 treatment means

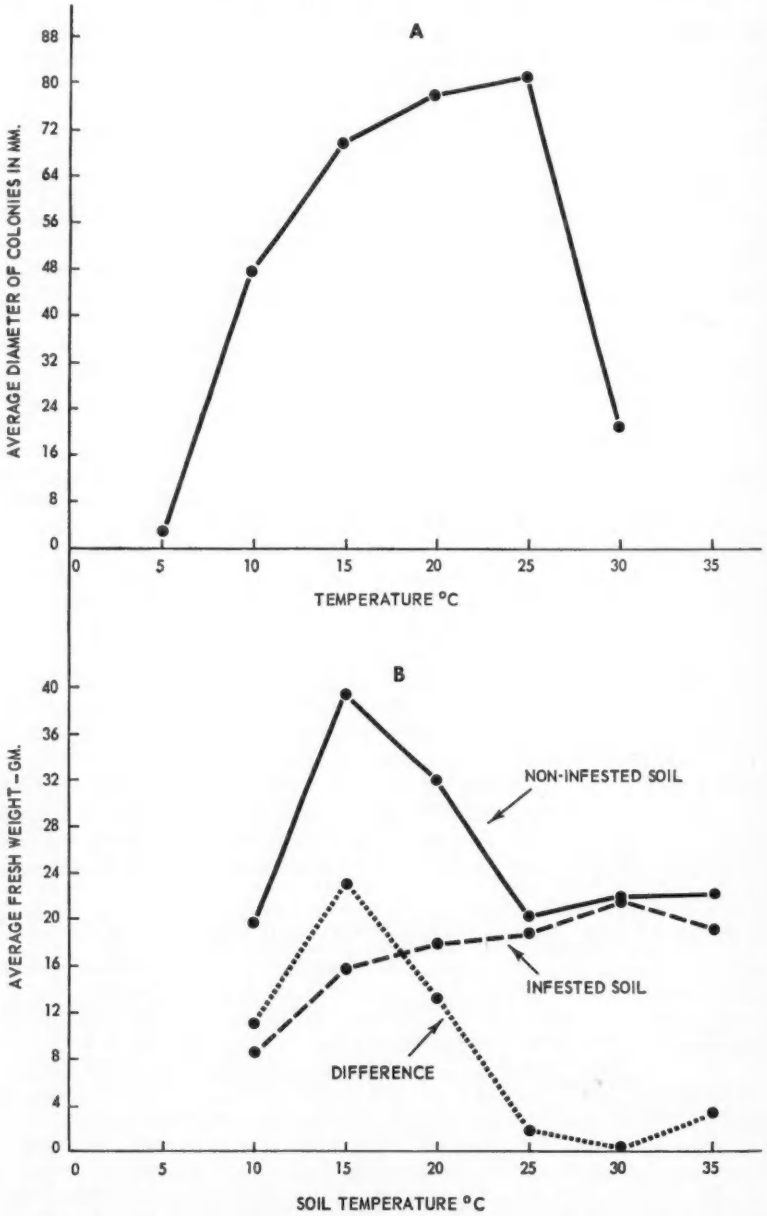

FIGURE 2. Effect of temperature on the growth of the pathogen and on the development of the host in infested and non-infested soil.

A. The average diameter of 4 colonies of *Phoma* sp. WD1 grown on potato dextrose agar for 2 weeks at 6 temperatures.

B. The average fresh weight of chrysanthemums grown for 9 weeks at 6 different soil temperatures.

nor did Srivastava's published description refer to these masses either on the infected host or in culture. Thus it seems best at present neither to identify *Phoma* WD1 with *P. chrysanthemicola* nor to specify the organism as a new species until the relationships have been further investigated.

EFFECT OF ENVIRONMENT ON DISEASE INCIDENCE AND SEVERITY

Soil Temperature

The variety "White Shasta" was grown in six constant temperature tanks in which both artificially infested and non-infested soil was maintained at 10°, 15°, 20°, 25°, 30° and 35°C. respectively. Two healthy rooted cuttings were placed in each container and the infested and non-infested series were replicated four times at each soil temperature. After 9 weeks, the fresh weight of the individual plants was recorded. The results are summarized graphically in Figure 2, B. The effect of temperature on the growth of isolate WD1 on potato dextrose agar plates over a 5-week period is shown in Figure 2, A.

The analysis of the data indicates that both the soil temperatures and soil treatments used in this experiment, as well as the interaction between these two factors, affect the growth of the variety "White Shasta". The curve expressing the difference between the mean fresh weight of the plants growing in infested and in non-infested soil at the various soil temperatures shows that the effect of the soil-treatment and the soil-temperature interaction is greatest at 15°C., which is optimum for the vegetative growth of the healthy host, and that the effect of this interaction diminishes sharply at 25°C., above which it is non-significant. Furthermore, it is clear that the growth rate of the pathogen at the different temperatures is not closely correlated with disease severity as measured by the mean fresh weight differences of the plants grown in infested and non-infested soil. The sharp reduction in disease severity in the 15° to 25°C. range suggests that the host becomes more resistant when grown at temperatures above optimum. It is also possible that changes in the infective capacity of the pathogen may change within this temperature range, even though the growth rate of the fungus in culture is unchanged.

Excessive Watering

An exploratory experiment was carried out to determine the effect of excessive watering on the incidence and severity of *Phoma* root rot in naturally infested greenhouse compost which was maintained at 50°, 55°, 60°, 65° and 70°F. This soil temperature range included the optimum temperature (60°F.) for the growth of florists' chrysanthemums. Two soil moisture levels were established ("wet" and "dry") by heavy and light watering. No attempt was made to calculate, adjust or maintain them accurately at any predetermined level. However, the soil in which the heavily watered plants were growing was never allowed to dry out and the surface of the soil was kept permanently moist, whereas the "dry" series of plants received only enough water to prevent wilting. Included in this experiment was a "dry" control in which the test variety was grown in naturally infested soil, which was previously steamed, in order that a com-

parison of the growth of the plants could be determined under approximately the same environment in infested and non-infested soil. Seven weeks after planting, the disease incidence and the fresh weight of the plants grown in these various soil treatments was recorded. These data are shown in Table 2.

Within the limits of this experiment, there appears to be no soil moisture level, no soil temperature, nor any interaction between these two factors which has significantly increased or decreased the pathogenic potential of the naturally infested soil or the susceptibility of the host to the disease. All of the plants in naturally infested soil either "wet" or "dry" and at any of the soil temperatures used in the test were severely infected. It can be seen that the fresh weight of these plants was reduced as much as 10 to 15 times that of plants grown in the same environment but in "dry" non-infested soil.

COMPARATIVE SUSCEPTIBILITY OF VARIETIES

In 1953, a small scale greenhouse trial was conducted with 26 varieties of commonly grown commercial florists' chrysanthemums to ascertain their

TABLE 3.—RELATIVE SUSCEPTIBILITY OF VARIOUS FLORISTS' CHRYSANTHEMUM VARIETIES TO PHOMA ROOT ROT

Variety	Size* type	Colour	Natural flowering date	Disease category
Pink Mistletoe	M-Inc.	Lavender	Dec. 5	
White Shasta	Anemone	White	Nov. 10	Extremely susceptible
Cream Shasta	Anemone	Cream	Nov. 10	
Yellow Shasta	Anemone	Yellow	Nov. 10	
Poinsettia	L. Single	Scarlet	Dec. 20	
Christmas Greeting	L. Pom.	Crimson	Dec. 15	
Yellow Seagull	M. Pom.	Light crimson	Nov. 1	
Blanche	—	White	—	
Gold Lode	M. Ref.	Light golden	Oct. 15	
Vibrant	M. Dec.	Deep lemon	Dec. 20	
White Mensa	Single	White	Nov. 5	
Masterpiece	L. Pom.	Rosewood	Nov. 12	Moderately susceptible
D.B. Orchid Queen	L. Inc.	Lavender	Nov. 10	
Belray	L. Pom.	White	Nov. 30	
Riviera	M. Dec.	Lavender rose	Dec. 15	
Ministrel	M. Pom.	Lavender	Dec. 12	
Shamrock	L. Dec.	Lemon	Dec. 15	
Gold Coast	M. Pom.	Deep lemon	Oct. 25	
Caroline Yossick	M. Pom.	Lavender	Oct. 20	
Frieda	—	—	—	
Little America	Anemone	White	Nov. 10	Slightly susceptible
Snowcrest	M. Pom.	White	Dec. 15	
Cameo	L. Pom.	Ivory	Dec. 5	
Silversmith	M. Dec.	White	Dec. 15	
Corsair	L.Pom.	Lemon	Dec. 12	
Indianapolis White	L. Inc.	White	Nov. 5	

* Pom. = Pompon Dec. = Decorative Inc. = Incurved
Ref. = Reflex L. = Large M. = Medium

relative susceptibility to *Phoma* root rot under similar growing conditions. The test included varieties which differed as much as possible in size, type, and colour of bloom and in natural flowering date.

Ten healthy rooted cuttings of each variety were grown in naturally infested soil in raised greenhouse benches. As a control, the same number of cuttings of each variety was benched in the infested soil which was steam sterilized before being used. Symptom records were taken at intervals during a 2-month period, after which time each variety was finally rated. Four empirical degrees of susceptibility were recognized: non-susceptible, slightly susceptible, moderately susceptible, and extremely susceptible. This classification was based on the over-all visible effects of the disease on any particular variety as compared with its healthy counterpart. The rating of the varieties tested is shown in Table 3.

The results indicate that, although there are varying degrees of susceptibility to the disease among the varieties used, no variety has been found to be completely immune to it. Furthermore, there appears to be no correlation between root rot susceptibilty and size, type, colour or natural flowering date of these varieties. It should be noted, however, that "White Shasta" and the cream and yellow sports of this variety are equally susceptible to the disease. Even though root lesions were extremely few on varieties classed as slightly susceptible, it was possible to isolate the fungus. In fact, it was usually easier to recover the pathogen more frequently from the roots of varieties in this category than from those which were classed as extremely susceptible, because plants in the latter group possessed only fragmentary roots which were in a badly deteriorated condition.

CONTROL

The preliminary control experiments with naturally infected soil showed that steam sterilization was effective in controlling the disease. However, many greenhouse operators do not have the necessary facilities for steam sterilization. As a result, experiments were conducted to learn whether a gaseous soil disinfestant or fungicidal soil amendment could be used to eliminate or reduce the disease.

Soil Fumigation

Both artificially infested (isolate WR2) and non-infested potting soils were treated with Dowfume MC-2 at a rate of 4 lb. per 100 sq. ft. of soil. The mixture was vaporized directly into these soils under a gas-proof polyethylene cover by means of a 280-ml. Arrow applicator. The soils were exposed to the gas for 40 hours and then were aerated for 96 hours before they were placed in steam sterilized 4-inch clay pots. Each pot was planted with a single healthy rooted cutting. Plants grown in soils treated with Dowfume MC-2 were compared with those in infested untreated soil. Each of the three treatments was replicated ten times. Nine weeks after planting, the disease incidence and the fresh weight of the plants were recorded. The data are shown in Table 4.

TABLE 4.—EFFECT OF METHYL BROMIDE SOIL APPLICATION ON DISEASE INCIDENCE AND GROWTH OF FLORISTS' CHRYSANTHEMUM

Soil treatment	Disease incidence	Growth mean fresh wt., gm.
Phoma WR2 infested, untreated	10/10*	5.3
Phoma WR2 infested, Dowfume MC 2 untreated	0/10	13.9
Non-infested, Dowfume MC 2 treated	0/10	13.8

*Numerator = No. plants infected
Denominator = No. plants inoculated

As Table 4 shows, root rot was eliminated by the use of Dowfume MC-2. Plants grown in the *Phoma*-infested soil were stunted, their roots were sparse and those which were present were seriously discoloured and decayed. On the other hand, the root systems in the treated soils were extensive and white and had no indication of infection. The similarity in mean fresh weight of the plants in the infested and non-infested soils treated with Dowfume MC-2 coupled with the general appearance of these plants, showed that the fumigant had no phytotoxic effects on the host.

Fungicide Soil Amendments

In an exploratory assay, employing a modified "paper-disk" plate method, it was shown that an aqueous suspension of Fermate (ferbam 76 per cent active ingredient) at a concentration of 1 in 100, and of Arasan (thiram 50 per cent active ingredient) at a rate of 1 in 100 and 1 in 1000 completely inhibited the growth of *Phoma* WD1. Furthermore, significant degrees of inhibition were obtained with both chemicals at rates up to and including 1 in 10,000. Subsequent experiments in which both chemicals were throughly incorporated with naturally infested greenhouse compost showed that neither fungicide reduced the severity of the disease sufficiently to warrant their use as a control measure. In another greenhouse trial, Brassicol (pentachloronitrobenzene 20 per cent) was mixed with artificially infested soil at a rate of 10 and 20 oz. per 100 sq. ft. These treatments also failed to reduce the incidence or severity of the disease.

DISCUSSION

During the past decade, it has become an increasingly popular practice for flower growers in Ontario to produce three crops of chrysanthemums a year in the same greenhouse bench. More recently, the newer "short disbud" method of chrysanthemum growing which can produce, in 1 square foot of bench area, 12 blooms with 18 to 24 inch stems in a matter of 13 weeks is being used by some progressive operators. This, of course, means that four crops a year can be grown in the same bench. Such production methods demand predetermined planting schedules with the shortest possible time-lapses between the harvesting of the flowering crop and the benching of the new one. Frequently the necessity for speed in replanting has resulted in the failure of the grower to steam, chemically disinfest, or replace his greenhouse soils for periods which often reach 2 years. The

constant growing of chrysanthemums in the same soil without steam or chemical treatments increases, for successive crops, the hazard of new root rot problems such as the one reported here and often intensifies some of the older established diseases caused by various other agents.

The pathogenicity tests reveal beyond any doubt that the fungus *per se* has the ability to induce a severe root rot. It is unlikely that this disease will be found affecting summer crops of susceptible varieties. Experimental and observational data indicate that soil temperature is an important factor conditioning infection. Unfortunately the optimum soil temperature for the growth of many florists' chrysanthemums and for the development of the disease approximate each other so that the manipulation of this limiting factor is not feasible during periods when the soil temperature falls normally within the range for the maximum disease development.

Phoma WD1 has a wide pH tolerance in culture. No significant difference in growth of this isolate was noted between pH 4 and 7. This fact suggests that changing the pH of the soil would have little effect on the reduction of the disease.

Growers who have been confronted with the *Phoma* root rot problem are convinced that excessive watering is one of the factors responsible for an increased severity of this disease in chrysanthemum ranges. They make a point of watering lightly during the winter months which in any event is advantageous. Under the conditions of the experiment reported in this paper, it would seem that their suspicions are groundless. However, the possibility arises that the inoculum potential of the naturally infested soil used in this test was so great that any difference which might exist in disease severity due to excess soil moisture was completely hidden.

At first, it appears strange that the only authentic cases of *Phoma* root rot have been restricted to the variety "White Shasta" and that natural infection of "Poinsettia", "Christmas Greeting", and "Pink Mistletoe", shown to be extremely susceptible in the host range studies, has never been seen or reported. A possible explanation of this phenomenon is that these latter varieties are not usually suitable for flowering in a year-round program and they have escaped the disease mainly because environmental conditions have been unfavourable for disease development at the time they have been grown. On the other hand, "White Shasta" is flowered continually by most growers because it grows well throughout the entire year and because it is possibly the best white anemone-type available. As a result of its continual use, crops of this variety, if planted in infested soil, are certain to be growing during periods in which conditions are optimum for infection.

Advantage should be taken of the fact that soil temperatures of 55° to 60°F., which are optimum for disease development, occur most frequently in the early months of the year. During this period no known susceptible variety should be planted in soil that has a history of this disease unless it has been thoroughly disinfested. The pathogen is believed to be spread mainly in contaminated soils. There is no evidence to suggest that the fungus is carried within cuttings taken from infected plants, nor has this organism been found on any above-ground part of greenhouse-grown chrysanthemums. The facts suggest that the survival of the pathogen is

dependent on its invasion of roots in soils which are continuously cropped with the host. This probability emphasizes the necessity for thorough eradicatory and sanitary measures following crops which are infected.

Finally, it appears that the pathogen is limited in its host range. Although the effect of the fungus on all the major floricultural crops is not known, the results of the limited host range studies and observations made in greenhouses in which the disease was a serious problem suggest that *Phoma* root rot will not likely be a threat to other greenhouse crops.

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OBSERVATIONS ON THE EFFECTS OF COPPER FUNGICIDES ON STRAWBERRY FOLIAGE IN CENTRAL NEW BRUNSWICK[1]

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ABSTRACT

In central New Brunswick a severe reddening of strawberry foliage, due to the premature formation of anthocyanin, has consistently occurred after late application of copper fungicides (in June and later). Experiments in 1956 indicated that the formation of anthocyanin was accelerated by successively later applications of copper fungicides. Anthocyanin did not increase any more rapidly in foliage treated with non-copper fungicides than in untreated foliage. Hence, the condition is apparently caused by the action of copper and may be averted by the use of non-copper fungicides in all applications made in June or later.

INTRODUCTION

The present recommendation in New Brunswick for the control of strawberry leaf spot, *Mycospherella fragariae* (Tul.) Lindau, is to spray with Bordeaux mixture. When the mixture is used on strawberries in the fruiting year in central New Brunswick, however, a definite discoloration of the foliage occurs. Foliage may vary in colour from red to purple within 3 weeks after the application. A similar discoloration normally occurs in unsprayed plots also but the change is more gradual and reddening does not become pronounced until early autumn. Following procedures outlined by Blank (1) and Robinson (3), tests of the extracted pigment have shown it to be an anthocyanin.

This is a report on the effects of copper fungicides on the increase of anthocyanin formation in strawberry foliage, and of the effect of time of application of a copper fungicide on the rate of formation of anthocyanin.

METHODS

In the spring of 1956 a plantation of Senator Dunlap strawberries in Queens County, New Brunswick, was divided into a 9 x 9 latin square, each plot measuring 8 by 10 feet. The treatments consisted of three non-copper and five copper fungicides, and the check. The materials used and their sources are shown in a footnote to Table. 1.

The anthocyanin content of the foliage was determined from one 10-gram sample taken from each plot immediately before, and at 3 weeks after, the treatment. Each sample consisted of approximately 12 leaves, free of leaf spot and picked at random from runner plants.

In another experiment, three plantations of Senator Dunlap strawberries were each divided into five 16 by 14 feet plots. One plot in each plantation was not sprayed and served as a control. Each of the other

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TABLE 1.—ANTHOCYANIN CONCENTRATIONS* IN STRAWBERRY FOLIAGE AFTER APPLICATION OF COPPER AND NON-COPPER FUNGICIDES

	On day of application**	Three weeks after application**
Copper fungicides		
Crag potato fungicide[1]	8.1	33.2
Bordeaux mixture (4–4–40)	7.1	45.3
Basicop[1]	6.9	58.5
C.O.C.S.[2]	7.4	80.3
Perenox[3]	8.8	89.6
Non-copper fungicides		
Parzate[2]	7.6	11.7
Glyodin[1]	7.2	13.2
Captan[2]	10.2	17.4
Control	10.7	14.3
Difference for significance at the 1 per cent level	9.3	13.5

*Percentages of the maxima in completely reddened foliage
**Difference between dates necessary for significance at the 1 per cent level = 12.28
[1]Obtained from Sherwin Williams Co., Montreal, Que.
[2]Obtained from Niagara Brand Spray Co., Burlington, Ont.
[3]Obtained from Canadian Industries Ltd., Halifax, N.S.

TABLE 2.—ANTHOCYANIN CONCENTRATIONS* IN NORMAL STRAWBERRY FOLIAGE, AND IN TREATED FOLIAGE, 3, 4, AND 5 WEEKS AFTER THE APPLICATION OF A BORDEAUX MIXTURE

Date	Normal	Weeks after application		
		3	4	5
May 17**	4.7	4.1	3.2	3.5
June 7**	4.1	7.4	19.5	27.3
June 14	3.8			
June 21	3.9			
June 28**	4.4	37.3	59.2	73.6
July 5	5.7			
July 12	6.7			
July 19**	6.7	58.7	60.9	71.5
July 26	8.9			
August 2	10.9			
August 9	11.4			
August 16	17.6			
August 23	50.7			
August 30	83.1			

*Means of 9 replications, as percentages of the maxima in completely reddened foliage
**Date of application of Bordeaux mixture (4–4–40)

plots received one application of a 4–4–40 Bordeaux mixture, spraying being done at 3-week intervals so that the first plot was sprayed on May 17 and the last one on July 19. The anthocyanin content of the foliage was determined from three 10-gram samples taken from each plot immediately before and at weekly intervals, beginning with the third week after the application.

For the purpose of relating all readings to a common standard, the anthocyanin content was measured as follows: A standard solution that closely matched the colour of an extract of anthocyanin from strawberry foliage was made by dissolving 0.030 grams of methyl orange in one litre of 1 per cent hydrochloric acid. This solution had the same optical density as an extract of anthocyanin from completely reddened foliage. Each 10-gram sample of foliage was macerated in a Waring blendor containing 200 millilitres of 1 per cent hydrochloric acid and 5 grams of Celite*. The extracts were filtered and the absorption was determined with a Klett-Summerson colorimeter with a green (500-570 m μ) filter, and expressed as a percentage of the optical density of the standard methyl orange solution. From this an estimate was made of the relative concentration of anthocyanin in the samples.

RESULTS

Table 1 shows that there was no significant increase in anthocyanin concentration in the plots sprayed with non-copper fungicides, but in the plots sprayed with copper fungicides the increase was significant at the 1 per cent level.

Table 2 shows that in the unsprayed plots the anthocyanin concentration increased slowly before the middle of August and then increased very rapidly, so that the foliage was almost completely reddened within the next 2 weeks. Applications of Bordeaux mixtures made before June 1 had no effect on anthocyanin formation; but there was a marked effect in the rate of formation in all plots sprayed at later dates.

DISCUSSION

The apparent association between the application of copper fungicides and the premature formation of anthocyanin indicates that copper influences the formation of the pigment. Further support is contained in the work of Edmondson and Thimann (2), who, in their study of deficiency effects and copper chelating substances, concluded that copper was necessary in the biogenesis of anthocyanin in *Spirodela* sp.

The data from the second experiment suggest that the later the date of application, the more rapid the increase in formation. This acceleration in rate of formation normally terminates, however, when the rate of formation begins to accelerate in unsprayed foliage.

At the time of these experiments the recommendation for control of insects and diseases of strawberries in New Brunswick included three applications of a 3–6–40 Bordeaux mixture, the first when the buds are beginning to form (mid-May), the second when the first green berries are beginning to form (early June), and the third immediately after harvest (early August). The data in this paper indicate that the premature reddening of the foliage can be averted by use of a non-copper fungicide instead of Bordeaux mixture in all applications made later than May.

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CEREAL SMUT RACES AND THEIR VARIABILITY[1]

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ABSTRACT

The study of physiologic specialization in cereal smuts, excluding bunt of wheat, involved subjecting 2880 smut collections and selected subcultures to pathogenicity tests on differential varieties of host plants. The results of these tests showed that (a) a smut collection may consist of a pure strain but more frequently of mixed or heterozygous strains; (b) repeated passage of a variable culture through selected hosts occasionally yielded a stable strain which may be called a race; (c) the majority of variable cultures continued variable through ten generations of selection. Covered smut of oats was the least variable; covered smut of barley, loose smut of oats and false loose smut of barley were progressively more variable. No stable strain or race has been isolated in the flower-infecting loose smut of wheat and of barley. Preliminary results of selfing some of the variable smut cultures indicate a possibility of obtaining races stable for pathogenicity on the differential hosts.

Following the discovery of physiologic specialization in cereal rusts (8, 21), studies on host specialization in other plant-pathogenic fungi were undertaken. Investigations on physiologic specialization in smut fungi began in 1919 when Kniep (13) noticed differences in the appearance of sporidial cultures of the anther smut of violet (*Ustilago violacea* Pers.). Two years later, Zillig (26), demonstrated that physiologic races of this smut could be differentiated on the basis of their ability to infect certain members of the Caryophyllaceae and not others. Investigations on other smut species were made by Faris (9) Reed (19) and others (7, 10, 11, 14, 15, 17, 24, 25) with the result that a number of races have been described (1, 2, 3, 12, 20, 22, 23) in each species that has been studied.

It is apparent that a knowledge of pathogenic types in smuts, their range of virulence and their distribution in a given area will facilitate the development of resistant varieties of host crops. These pathogenic types have been designated as physiologic races, a term created by rust investigators to describe pathogenically distinct, dicaryotic uredial clones. These rust clones can be kept in culture indefinitely without their passing through a sexual phase and, therefore, will remain stable entities even if they are heterozygous for many pathogenic traits. On the other hand, smut cultures must pass through a sexual phase after each infective generation and therefore, unless they are homozygous for the pathogenic characters being studied, they cannot be regarded as stable physiologic races. Studies on inheritance of reaction to smuts in cereal crops are complicated by the variability of smut cultures. The extent of this variability is of particular interest to persons concerned with the development of cereal varieties resistant to smuts. It is the purpose of this paper to record the infectivity of some smut cultures that have been studied, generation after generation, to determine their variability on differential hosts and to discuss the significance of this variability in relation to the breeding of smut-resistant cereals.

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METHODS AND MATERIALS

For seedling-infecting smuts of oats and barley the Waring-Blendor method (18) proved the most suitable for large scale tests of pathogenicity. This method was made even more effective when the inoculated seed was stored in a cool humid place for about a month before seeding. The average percentage of infection in a susceptible variety of barley after storage was 72, as compared to 53 when dry or wet spores were applied to dehulled seed just before seeding. For flower-infecting loose smut of wheat and of barley the improved partial vacuum method (5) was used until 1955, when the mechanical pump was developed (6) to avoid the variation in the speed and force of pumping between different pump operators and during different hours of the day. The latter has been used for loose smut inoculations during the past 3 years.

Smut collections were obtained from all provinces of Canada, although the majority were collected in the three Prairie Provinces. Each collection was subjected to a pathogenicity test on a uniform set of differential host varieties. Many collections were re-tested, some as many as 34 times. The inoculum of subcultures for these re-tests was obtained from individual differential hosts. Single-spore cultures from a few of the most variable collections were also tested with the hope that a stable entity could be isolated. During the 10 years of study 1219 field collections of the various smuts were tested and, together with re-tests, 2880 smut cultures were subjected to pathogenicity tests on differential hosts. These numbers included 111 field collections and 349 subcultures of *Ustilago avenae* (Pers.) Rostr.; 353 and 311 of *U. hordei* (Pers.) Lagerh; 301 and 178 of *U. kolleri* Willie; 141 and 350 of *U. nigra* Tapke; 222 and 294 of *U. nuda* (Jens.) Rostr., and 91 and 179 of *U. tritici* (Pers.) Rostr.

Besides the field collections, selected subcultures, and single-spore cultures, 290 selfed lines obtained from paired monosporidial cultures of the four seedling-infecting smuts of oats and barley were subjected to pathogenicity tests on differential host varieties.

The differential hosts for seedling-infecting barley smuts were the same as those used by Tapke (22, 23) and for oat smuts were the same as those used by Holton and Rodenhiser (12) with the addition of the varieties Mabel and Beacon. For the loose smut of wheat, Oort's (17) hosts were used, except that two of his winter wheat varieties, Bersee and Von Rumker's Dikkop, were replaced by Kota and Red Bobs and two additional varieties, Reward and Pentad, were added to the group. A set of 30 varieties of barley was used for the loose smut collections during the first 3 years and then 10 of these were selected as differential hosts (3).

Seed of differential hosts was obtained originally from the investigators who selected the sets. The varieties added or substituted and those selected by the author for differentiation of loose smut of barley were obtained through the Laboratory of Cereal Breeding, Winnipeg, Manitoba. Before inoculation with the seedling-infecting smuts the seed was treated in 0.13 per cent formaldehyde solution for 30 minutes, then washed, dried and packaged, with approximately 12 grams of seed per packet. The process of inoculation consisted of dumping seed from a packet into a spore suspension in a running Waring Blendor and agitating it for 12 to 25 seconds,

the time depending on the toughness of the hulls. More injury to the seed resulted if it was dropped into the spore suspension before the blendor was started. The seed was then strained, divided into two equal parts, and packaged. After the seed had dried sufficiently so as not to mould or germinate, the packages were stored in a cool, humid room until seeding time. Seeding was usually started when soil temperature at a depth of 2 inches had reached 60°F. The inoculated seed of each set of differentials in duplicate was seeded in 6-foot rows, spaced 12 inches apart. The results were based on the mean percentage of smutted heads in the two rows of each differential host.

The seed of differentials for loose smuts of wheat and barley was treated with an organic-mercury seed disinfectant and, for plants to be inoculated, was seeded in 5-foot rows, spaced alternately 12 inches and 24 inches apart. Inoculated seed for smut counts was seeded in single 6-foot rows, spaced 12 inches apart.

RESULTS

Only a few races in the seedling-infecting smuts of oats and barley, namely Races 1 and 3 of *U. kolleri*, 1 and 5 of *U. avenae*, 6 and 7 of *U. hordei*, and Race 4 of *U. nigra*, were obtained in a more or less stable form. Occa-

TABLE 1.—MEAN PERCENTAGE INFECTIONS ON DIFFERENTIAL HOSTS OF OAT SMUT CULTURES AND SUBCULTURES, SHOWING EXTENT OF VARIABILITY BETWEEN DIFFERENT SELECTIONS

Differential hosts	Host no.	*U. kolleri* 45–6 (Dafoe, Sask.) Origin and generation of subculture selections[1]													
		F	2 10	2 12	3 12	3 3	4 12	4 10	5 12	6 12	7 12	8 12	9 12	*	*
Anthony	1	29	22	55	61	33	65	42	57	45	19	68	80	72	59
Black Diamond	2	7	14	23	33	11	34	17	29	10	6	28	73	21	29
Victory	3	10	8	27	46	20	57	27	30	14	11	59	52	49	44
Gothland	4	0	0	0	0	0	0	0	1	0	0	0	0	57	0
Monarch	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Fulghum	6	2	0	2	0	1	1	1	1	2	0	0	5	7	4
Black Mesdag	7	0	0	0	0	0	0	0	1	0	0	0	1	2	0
Camas	8	0	0	0	0	0	0	2	2	0	0	0	0	0	0
Nicol	9	0	0	0	0	0	0	1	0	0	0	0	1	0	0
Atlantic	10	3	1	0	4	0	1	1	1	0	0	0	0	0	0
Mabel	11	0	32	1	1	0	0	0	0	0	0	0	0	55	0
Beacon	12	44	0	67	64	30	84	34	56	55	49	80	59	80	73
Race identified	[2]	K1?	K1?	K1	K1	K1	K1	K1	K1	K1	K1?	K1	K1	K3	K1

Differential hosts	Host no.	*U. avenae* 45-32 (Gilbert Plains, Man.)													
		F	2 11	3 11	4 12	5 9	6 1	7 1	7 9	8 9	8 10	9 9	*	*	*
Anthony	1	26	20	40	56	53	76	57	59	93	83	73	81	81	10
Black Diamond	2	3	14	12	39	8	34	4	10	79	14	49	17	50	9
Victory	3	10	32	22	53	55	66	54	66	80	42	68	68	76	10
Gothland	4	0	0	0	18	1	75	1	4	0	0	0	83	5	6
Monarch	5	0	0	0	0	0	0	0	0	0	0	0	2	1	44
Fulghum	6	0	0	0	6	5	5	1	0	0	2	3	3	8	76
Black Mesdag	7	0	0	1	1	0	0	0	5	1	1	0	0	2	0
Camas	8	0	0	1	3	2	0	14	3	3	0	3	2	0	0
Nicol	9	0	1	12	9	18	2	21	20	19	10	19	0	5	7
Atlantic	10	0	5	3	0	0	0	1	0	0	0	0	0	1	0
Mabel	11	23	23	55	65	76	77	70	79	86	85	72	74	79	22
Beacon	12	1	0	0	25	3	84	0	11	0	1	16	80	83	50
Race identified	[2]	+	A1D	+	A5	+	A5	+	+	+	+	+	A5	A1C	+

—continued on next page

TABLE 1.—MEAN PERCENTAGE INFECTIONS ON DIFFERENTIAL HOSTS OF OAT SMUT CULTURES AND SUBCULTURES, SHOWING EXTENT OF VARIABILITY BETWEEN DIFFERENT SELECTIONS—*continued*

Differential hosts	Host no.	*U. avenae* 47–4 (Sperling, Man.) Origin and generation of subculture selection[1]												
		F	1^5	2^5	3^5	4^5	4^{11}	5^{11}	5^5	6^5	6^{12}	7^5	8^5	9^6	10^{10}
Anthony	1	65	10	19	32	44	21	40	48	53	64	71	85	54	79
Black Diamond	2	10	3	9	21	35	18	14	26	15	33	38	74	55	50
Victory	3	14	2	10	28	19	16	22	26	33	31	39	50	27	47
Gothland	4	15	1	1	13	28	25	36	13	7	9	27	25	30	25
Monarch	5	8	12	26	58	60	0	0	68	60	42	99	92	78	90
Fulghum	6	1	0	1	3	3	1	2	8	0	4	10	9	5	6
Black Mesdag	7	0	0	0	1	0	1	0	0	0	0	0	1	0	2
Camas	8	0	0	0	0	0	41	55	0	3	0	4	0	0	1
Nicol	9	0	0	1	8	11	0	2	5	0	1	6	4	1	6
Atlantic	10	0	0	2	13	0	0	0	0	0	0	3	0	1	0
Mabel	11	64	10	14	58	73	36	40	57	72	76	83	82	70	95
Beacon	12	38	12	31	61	62	5	7	55	51	64	49	80	59	91
Race identified	[2]	A5	+	+	A20?	A19?	+	+	A6	A6?	A6?	A6?	A6	A6	A6

Differential hosts	Host no.	*U. avenae* 49-16 (Fredericton, N.B.)													
		F	1^3	2^6	3 s.sp.	3^9	3^8	4^1	5^8	5^{11}	7^6	*	*	*	*
Anthony	1	21	37	75	49	46	28	94	74	78	64	68	43	64	81
Black Diamond	2	16	35	23	54	9	22	14	60	48	52	33	29	62	46
Victory	3	8	43	33	23	28	20	44	42	90	61	23	27	53	45
Gothland	4	27	60	1	62	0	39	0	68	0	0	0	0	74	66
Monarch	5	0	1	0	1	0	0	0	15	0	0	8	10	0	0
Fulghum	6	0	4	2	3	3	1	0	1	3	5	0	8	0	4
Black Mesdag	7	1	2	0	0	0	0	0	0	25	5	0	0	6	0
Camas	8	22	41	2	26	0	41	0	64	0	0	50	0	3	65
Nicol	9	7	17	11	9	14	9	4	17	4	26	0	0	2	2
Atlantic	10	1	9	0	0	0	0	0	0	2	1	0	0	0	2
Mabel	11	25	61	52	68	67	51	0	88	77	80	44	63	64	58
Beacon	12	13	36	5	42	2	1	82	8	3	4	0	50	78	6
Race identified	[2]	+	+	+	+	+	+	Alb	A7	+	+	A2	A4	A5	+

[1]The first column of percentage figures represents the test of the original field culture; subsequent columns represent different re-tests with selected subcultures from differential hosts, e.g. 10 = selection from Atlantic, 12 = selection from Beacon, etc.; s.sp. = single spore subculture; and * = selfed lines obtained from paired monosporidial cultures. Superscript numbers indicate generations in which subcultures were selected.

[2]The letter and number at the bottom of each column indicate physiologic race or a biotype, according to the reaction on differentials, e.g. K1 = Race 1 of *U. kolleri*; A1 = Race 1 of *U. avenae*.; + indicates an undescribed race.

sional field collections consisted of one of the above-mentioned races which maintained its pathogenic behaviour up to ten generations. More frequently field collections consisted of a mixture of two or more races from which a stable entity or entities could be obtained by selection and re-selection through several generations. The majority of smut collections, however, consisted of heterogeneous or heterozygous strains which could not be separated into stable entities by repeated passage through selected hosts for as many as ten generations. Even single teliospore cultures of the unstable strains continued to vary from generation to generation. A few examples of the extent of such variability are shown in Tables 1 and 2.

The least variability occurred in the covered smut of oats, *U. kolleri*. Even in this smut some cultures varied in their pathogenicity on certain differential hosts. Culture *U. kolleri* 45-6 (Table 1), selected from Atlantic oats, was virulent on Mabel and not on Beacon, while selections from other hosts were virulent on Beacon but not on Mabel. The factor for

pathogenicity on Mabel was carried through several generations on Beacon without expression but some selfed lines obtained from paired monosporidial cultures were virulent on both Mabel and Beacon. Similarly, the factor for pathogenicity on Gothland appeared in some selfed lines although subcultures through ten generations of selection had not attacked this variety.

The loose smut of oats, *U. avenae*, showed much more variability. Culture *U. avenae* 45-32 became a typical Race 1D in second generation sub-culture from Mabel, but in subsequent subcultures its virulence on Nicol increased to the extent that it no longer resembled race 1D. Subcultures from Beacon selected Race 5 and subcultures from Nicol yielded an unidentified race or races. The selfed lines of this smut culture yielded Races 1C, 5 and an undescribed race or strain. The latter with its high virulence on Fulghum was unexpected, since none of the subcultures

TABLE 2.—MEAN PERCENTAGE INFECTIONS ON DIFFERENTIAL HOSTS OF BARLEY SMUT CULTURES AND SUBCULTURES, SHOWING EXTENT OF VARIABILITY BETWEEN DIFFERENT SELECTIONS

Differential hosts	Host no.	*U. hordei* 46-3 (Upper Mangeville, N.B.)													
		Origin and generation of subculture selections[1]													
		F	2 3	3 4	4 4	4 7	6 3	7 3	9 3	9 5	10 7	*	*	*	*
Excelsior	1	0	0	0	0	0	0	0	4	0	0	0	0	0	0
Hannchen	2	27	22	24	32	16	31	28	36	22	19	32	50	47	30
Himalaya	3	6	6	16	16	6	24	28	24	24	27	8	50	41	0
Lion	4	13	8	20	25	10	7	28	24	14	5	24	26	57	9
Nepal	5	5	10	2	8	8	0	2	42	18	26	2	0	10	6
Odessa	6	59	46	56	63	52	49	30	48	50	3	18	75	49	58
Pennier	7	0	0	0	0	0	0	0	14	0	0	0	0	0	3
Trebi	8	47	6	26	32	14	49	14	36	34	29	50	17	59	2
Race identified	[2]	6	+	+	+	6	+	+	10	11	+	6	+	11	1

Differential hosts	Host no.	*U. hordei* 49-64 (Libau, Man.)													
		F	1 1	2 8	3 3	4 1	4 2	5 5	5 1	5 2	6 3	7 3	7 6	*	*
Excelsior	1	3	15	0	22	10	12	12	18	8	6	22	22	34	0
Hannchen	2	42	17	34	24	12	16	50	4	16	11	14	41	47	44
Himalaya	3	27	12	24	32	30	26	44	14	6	6	26	30	45	25
Lion	4	26	9	19	24	8	10	18	4	4	10	12	8	33	41
Nepal	5	21	37	3	32	40	48	30	56	42	57	27	39	43	0
Odessa	6	41	28	25	26	42	30	36	6	10	6	35	63	43	85
Pennier	7	4	1	0	0	0	0	2	0	0	0	0	0	0	0
Trebi	8	3	0	0	0	0	0	0	0	0	0	6	0	0	0
Race identified	[2]	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Differential hosts	Host no.	*U. nigra* 44-27 (Bowsman, Man.)													
		F	4 4	5 4	6 4	6 1	7 1	7 6	8 1	9 8	9 3	10 1	11 8	11 1	*
Excelsior	1	7	6	5	6	18	30	0	42	0	40	39	0	47	52
Hannchen	2	4	3	4	14	2	0	0	0	0	0	0	0	1	0
Himalaya	3	3	3	3	2	2	0	0	0	2	0	1	0	2	3
Lion	4	1	7	4	12	6	16	2	24	0	8	13	7	11	23
Nepal	5	18	24	23	8	24	44	24	22	29	36	41	31	41	45
Odessa	6	29	34	25	32	22	38	11	34	20	28	46	42	54	37
Pennier	7	1	1	6	8	0	0	0	2	1	0	0	0	0	0
Trebi	8	2	2	7	4	4	0	9	2	20	7	1	21	1	0
Race identified	[2]	9	9	9	10	+	+	9?	+	+	+	+	+	+	+

—continued on next page

TABLE 2.—MEAN PERCENTAGE INFECTIONS ON DIFFERENTIAL HOSTS OF BARLEY SMUT CULTURES AND SUBCULTURES, SHOWING EXTENT OF VARIABILITY BETWEEN DIFFERENT SELECTIONS—*continued*

Differential hosts	Host no.	*U. nigra* 45–21 (Ontario)													
		Origin and generation of subculture selections[1]													
		F	2 3	2 7	4 7	5 2	6 3	7 4	7 5	7 7	9 5	9 3	9 7	*	*
Excelsior	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Hannchen	2	1	12	8	6	3	4	6	8	14	15	7	0	34	43
Himalaya	3	5	10	12	44	41	27	4	14	48	30	30	6	49	36
Lion	4	0	0	0	0	0	5	0	8	10	3	4	9	0	45
Nepal	5	18	12	28	30	30	45	2	18	52	12	27	6	52	36
Odessa	6	13	42	30	26	29	17	10	38	24	40	43	24	36	31
Pennier	7	2	24	17	26	14	8	0	22	44	13	13	0	51	31
Trebi	8	4	10	2	12	16	5	0	14	26	13	14	0	30	13
Race identified	[2]	9	+	+	+	+	+	1	+	+	+	+	1	+	+

Differential hosts	Host no.	*U. nigra* 49-6 (Wembley, Alta.)													
		F	1 1	1 7	2 4	3 3	4 3	4 1	5 1	5 3	6 8	*	*	*	*
Excelsior	1	29	24	10	31	27	27	23	33	18	32	35	0	0	16
Hannchen	2	32	8	16	30	6	0	7	20	0	0	31	29	25	0
Himalaya	3	35	14	20	46	30	5	23	21	10	9	36	0	22	18
Lion	4	31	32	6	19	18	18	12	15	8	6	28	19	0	33
Nepal	5	29	45	11	64	55	16	23	35	21	25	43	0	18	33
Odessa	6	55	43	16	40	35	15	12	11	44	18	29	36	0	35
Pennier	7	5	0	8	49	0	3	3	2	0	0	0	0	0	0
Trebi	8	41	32	16	46	11	11	5	16	16	15	40	45	13	5
Race identified	[2]	+	+	+	13	+	8	+	6	+	+	6	4	+	+

Differential hosts	Host no.	*U. nigra* 49-7 (Nisku, Alta.)													
		F	2 8	3 8	3 1	3 2	3 4	3 s.sp.	4 5	4 7	7 2	8 8	*	*	*
Excelsior	1	2	4	2	20	1	12	1	33	12	0	20	4	17	0
Hannchen	2	48	10	35	5	22	60	51	2	1	15	1	40	0	0
Himalaya	3	3	2	1	35	2	35	0	20	8	9	2	9	7	0
Lion	4	15	13	20	8	9	18	4	13	7	6	5	22	3	5
Nepal	5	3	7	6	32	5	30	4	42	39	3	41	8	9	10
Odessa	6	55	23	40	32	21	51	34	19	16	24	5	20	29	0
Pennier	7	3	2	0	13	0	13	0	0	0	0	1	0	0	0
Trebi	8	30	26	46	44	37	38	39	26	24	15	8	12	11	0
Race identified	[2]	4	4	4	+	2	13?	2	+	+	2	+	4	+	+

[1]The first column of percentage figures represents the test of the original field culture; subsequent columns represent different re-tests with selected subcultures from differential hosts, e.g. 10 = selection from Atlantic, 12 = selection from Beacon, etc., s. sp. = single spore subculture, and * = self lines obtained from paired monosporidial cultures. Superscript numbers indicate generations in which subcultures were selected.

[2]The letter and number at the bottom of each column indicate physiologic race or a biotype according to the reaction on differentials, e.g. K1 = Race 1 of *U. kolleri*, A1 = Race 1 of *U. avenae;* + indicates an undescribed race.

attacked this variety to any appreciable extent. Culture *U. avenae* 47-4 was variable, particularly on Gothland, Monarch and Camas. Selection through eight generations yielded a more or less stable Race 6, while other strains of this culture continued to show variability. Culture *U. avenae* 49-16 fluctuated in its pathogenicity on Gothland, Monarch, Black Mesdag, Camas, Nicol and Beacon. The 11 selfed lines of this culture that have been tested fall into four distinct races.

Cultures of the covered smut of barely, *U. hordei*, although more variable than those of the covered smut of oats, showed some stable strains. Race 6 was obtained a number of times in a relatively pure state from field

collections while Race 7 was readily isolated by selection through a few generations. Other cultures of this smut were extremely variable. Culture *U. hordei* 46-3 (Table 2) varied on a number of differentials but particularly on Himalaya and Nepal. Race 6 was isolated from this culture in the fifth generation of selection but other strains remained variable through ten generations. The heterozygosity of this culture was demonstrated by selfed lines which indicate at least four different races or strains. Culture *U. hordei* 49-64 varied mainly on Excelsior and Nepal varieties. Selection from Himalaya through three generations yielded a relatively stable entity which also appeared in some selfed lines. The two races indicated by selfed lines of this culture have not been previously described.

In the false loose smut of barely, *U. nigra*, there was more variability than in other seedling-infecting smuts of oats and barley. With the exception of Race 4 which remained stable, all collections of this smut yielded more or less variable strains. In culture *U. nigra* 44-27, selection through several generations from Excelsior yielded a stable strain which also appeared in some selfed lines. Culture *U. nigra* 45-21 yielded two more or less stable strains which reappeared in selfed lines but this required selection through eight generations. The other two cultures of this smut, shown in Table 2, remained variable through ten generations. The heterozygosity of these two cultures is indicated by the appearance in each of them of three or more distinct races or strains among selfed lines.

Selections of subcultures from individual differential hosts were also made in the collections of loose smut of wheat and of barlye (*U. tritici* and *U. nuda*). Because two crop seasons are required for each generation of the smut, only five to ten subcultures have so far been tested. From these relatively few re-tests no stable culture of loose smuts has yet been isolated.

DISCUSSION

Most of the cereal smut cultures are variable entities probably because there is segregation and recombination of genetic factors in each generation of the smut fungi. The variability, however, is not the same in all species. In the covered smut of oats variability occurs only in some collections and, even in these, stable cultures can be obtained by selection of inoculum from specific hosts through a number of generations. Covered smut of barlye is somewhat more variable than the covered smut of oats but stable cultures of this smut may also be selected, although in some collections it may require selection through many generations. Loose smut of oats is still more variable. In some cultures selection through a number of smut generations yields what appears to be a fairly stable culture. Selection in such a culture through another host, however, may yield a culture with different pathogenic behaviour. False loose smut of barley is the most variable of the seedling-infecting smut fungi of coarse-grains. Only one race, Race 4, was maintained through at least ten generations without any significant changes. This race happens to be the most prevalent in Canada. Race 9 was another one isolated and maintained through several generations without significant variation but inbreeding of this culture by paired monosporidial lines from the same promycelium accentuated the

variation on certain differential hosts. In some other cultures of this smut, selection through ten generations did not yield any indication that a stable culture could be isolated.

The cause of the variability in many cereal smut cultures has not been established. If the variability is due to mutations, then stable entities would not be obtained either by selection or inbreeding. The fact that at least one race in the most variable species, *U. nigra*, remained constant for ten generations indicates that mutations are not very frequent, unless the frequency of mutation varies between races of the same species. If the variability is due to a mixture of races in a given smut collection, then it should be possible to separate such a mixture by selection from individual differential hosts for two or three generations. On the other hand, if an unstable smut race constitutes a genotype heterozygous for pathogenicity with avirulence dominant and with a complement of modifying factors influencing pathogenicity on various differential hosts, it would be difficult to select a genotype stable on differential hosts. The results of selection and selfing of certain smut races indicate that virulence is commonly recessive. This may well explain the variability in many smut races and their ability to adapt themselves to previously resistant varieties of their host crops.

Each biotype of a smut culture must possess several loci responsible for virulence and avirulence on a group of differential hosts. It is likely that in some cases two or more loci are concerned with pathogenicity on a specific host variety. For any single locus three genotypes are possible: aa, Aa and AA, the first virulent on a specific host, the second avirulent but with some survival possibility and the third avirulent with perhaps no survival value. Then for any individual locus, if the heterozygote does have a chance for survival, however small, the dominant avirulent gene will be carried along with the recessive genes for virulence. For any individual locus the final proportions of dominant and recessive genes in the culture will depend on the host that is being used. For n loci 3^n genotypes are possible. After culturing for a few generations on different hosts, the genetic constitutions of the subcultures will not be identical. This is according to the Hardy-Weinberg law. Survival possibility of more than one genotype for a number of loci is clearly indicated by the occurrence of pathogenicity in some selected subcultures and selfed lines on hosts previously resistant (Tables 1 and 2). The probability of a substantial change in a selected subculture and particularly in selfed lines is enhanced because of the small population that is involved. For any one locus in selfed lines one-quarter of such lines would have the homozygous recessive genotype, virulent on a specific host.

From the preliminary tests with selfed lines of a number of variable smut cultures it seems that development of races homozygous for pathogenicity on differential hosts is a definite possibility. If this is substantiated by further studies, then it would take less time and effort to identify smut races by selfing variable cultures than by selection of inoculum from specific differential hosts. Such stable races would be of great importance in testing inheritance of resistance or susceptibility in varieties of cereal crops.

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A PHOTOELECTRIC DEVICE FOR MEASUREMENT OF LEAF AREAS[1]

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ABSTRACT

A device for the rapid measurement of leaf areas is described. Leaves are placed between a fluorescent light source, high in the red spectrum, and a photocell covered with an F29 red light filter. The reduction in output of the cell, as measured by a galvanometer, is interpreted in terms of area. The device has been successfully used in a genetic study of leaf size in birdsfoot trefoil.

INTRODUCTION

To carry out many investigations in the plant sciences a rapid and accurate means of determining the area of leaves is a necessity. The number of measurements to be made, the wide variations in leaf shape which occur, and the accuracy required often preclude the use of such laborious methods as printing the leaf outlines on light sensitive paper and measuring them with a planimeter or calculating area from linear measurements using geometric formulae. Realizing this, a number of workers (1, 2, 3, 4, 5, 6) have built and described photoelectric devices which they used in studies concerned with leaf size measurements. The principle involved is the direct relationship which exists between the area of a leaf placed between a light source and a photocell and the reduction in output of the cell due to part of the light being blocked off.

In the construction of one of these devices there are two main requirements to consider. Irradiation of the specimen stage should be as uniform as possible in order that "position effects" will be negligible and some provision should be made to compensate for the fact that most leaves are not entirely opaque but transmit some yellow-green light. In the device to be described here, these requirements, within acceptable tolerances, have been met by the use of a fluorescent light high in the red spectrum and a red light filter, features which have not been used in devices previously described.

CONSTRUCTION DETAILS

The structure of this device is shown in Figure 1.

The cabinet consists of two parts and is built of $\frac{3}{4}$-inch plywood. The lower part, the "light box", is a 26-inch cube, inside measurement. This dimension, which is not critical, was determined by the space required to accommodate the fluorescent lamps on the under side of the box lid. The "light box" is painted white on the inside and has a piece of white blotting-paper tacked on the floor to improve diffusion of the light reflected through the specimen stage. Overheating of the lamps is prevented by the

[1]Joint contribution from the Forage Crops Division and the Field Husbandry, Soils and Agricultural Engineering Division, Canada Department of Agriculture, Ottawa, Ont.

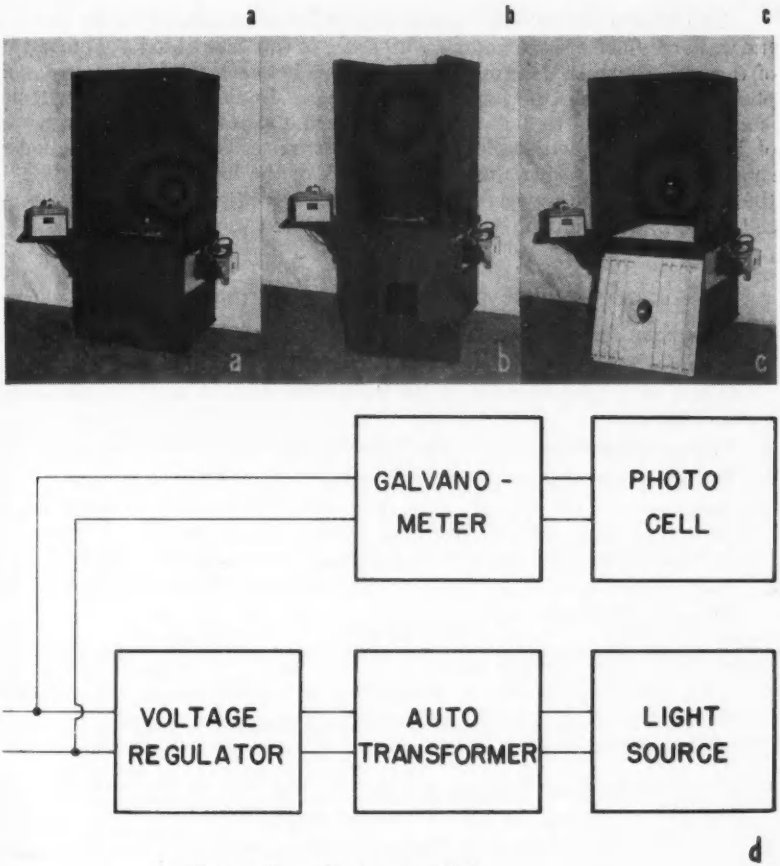

FIGURE 1. a. Device assembled.
b. Cupola front and top removed.
c. Light box lid removed.
d. Circuit diagram.

circulation of air through slots cut in the walls. The "light box" lid has a hole 6 inches in diameter cut exactly in its centre. Fitted into this hole is a cylinder made of white blotting-paper which projects 1 inch below the fluorescent lamps.

The specimen stage consists of a piece of plate glass, 10" x 12" x 3/16", mounted in a metal frame on the upper side of the lid and centred over the 6-inch hole. Beneath this is placed a piece of 22-gauge galvanized iron of the same length and width, painted black and having a hole $5\frac{3}{4}$ inches in diameter cut in its centre. Projection of the light through the hole in this thin plate ensures a well defined beam. A hinged glass plate, 8" x 10" x 3/16", is mounted over the specimen stage and isused to hold the leaves flat.

The second part of the cabinet consists of a cupola, 26″ x 26″ x 36″, the walls of which are continuous with those of the "light box". The height of the cupola, which determines the distance between the light source and photocell, should not be less than 36 inches. In the front of the cupola is a "working port" and above this is housed a series of four baffles, made of ½-inch plywood with holes cut in the centres. The size of these holes should be slightly larger than the diameter of the light beam in use. On the under side of the cupola lid and exactly in the centre is mounted a photocell behind a red light filter, the latter being used to correct for leaf transparency.

APPARATUS AND SPECIFICATIONS*

1. *Fluorescent Lamps*—8, F20T12, pink, 20 watts each.
2. *Selenium Photocell*—International Rectifier Corp., 6″ diam., rated at 950μ a. at a light intensity of 100 ft. candles with an external resistance of 100 ohms.
3. *Voliage Regulator*—250 VA, 110V, 60 cycles.
4. *Autotransformer*—3 amps, O–130V output, 60 cycles.
5. *Galvanometer*—Kipp A–70, scale deflection of 1 mm. at 1 metre for a current of 0.001 microamps, and internal resistance of 80 ohms, an external resistance of 100–2000 ohms and five sensitivity ranges.
6. *Light Filter*—Canadian Kodak, F29, deep red, spectral transmission 610 mμ to the infra red.

*The mention of specific instruments or trade names is made for purpose of identification only and does not imply any endorsement by the authors.

TABLE 1—CALIBRATION DATA (GALVANOMETER READINGS IN SCALE UNITS), SHOWING EFFECT OF POSITION OF CALIBRATION PIECE IN A LIGHT BEAM 5¾ INCHES IN DIAMETER

Known area[1] (sq. cm.)	Position of calibration piece in light beam							
	Centre	Left	Right	Front	Rear	Average	Range	%Error[2]
5	7.4	7.3	7.5	7.5	7.6	7.5	0.3	3.9
10	14.4	14.1	14.1	14.3	14.2	14.2	0.3	2.1
15	21.8	21.6	21.5	21.5	21.8	21.6	0.3	1.4
20	29.2	29.0	28.9	28.9	29.0	29.0	0.3	1.0
25	36.2	35.9	35.9	35.9	36.0	36.0	0.3	0.8
30	43.5	43.2	43.1	43.1	43.3	43.2	0.4	0.9
35	50.5	50.3	50.3	50.3	50.3	50.3	0.2	0.4
40	57.4	56.8	56.8	56.9	57.0	57.0	0.6	1.0
45	64.4	64.0	64.0	64.0	64.2	64.1	0.4	0.6
50	71.6	71.3	71.3	71.2	71.4	71.4	0.4	0.6
55	78.1	77.9	77.9	77.8	77.8	77.9	0.3	0.4
60	85.0	85.0	85.0	84.9	84.9	85.0	0.1	0.1
65	92.0	91.8	91.8	91.7	91.9	91.8	0.3	0.3
70	98.4	98.1	98.1	98.0	98.4	98.2	0.4	0.4
75	105.5	105.4	105.3	105.3	105.4	105.4	0.2	0.2
Average	57.0	56.8	56.8	56.8	56.9	56.8	0.2	0.4

[1]Calibration pieces were carefully cut from black fibreboard approximately 0.6 mm. in thickness.
[2]Expressed as per cent of the maximum reading in the series which, in most cases, is that for the centre position.

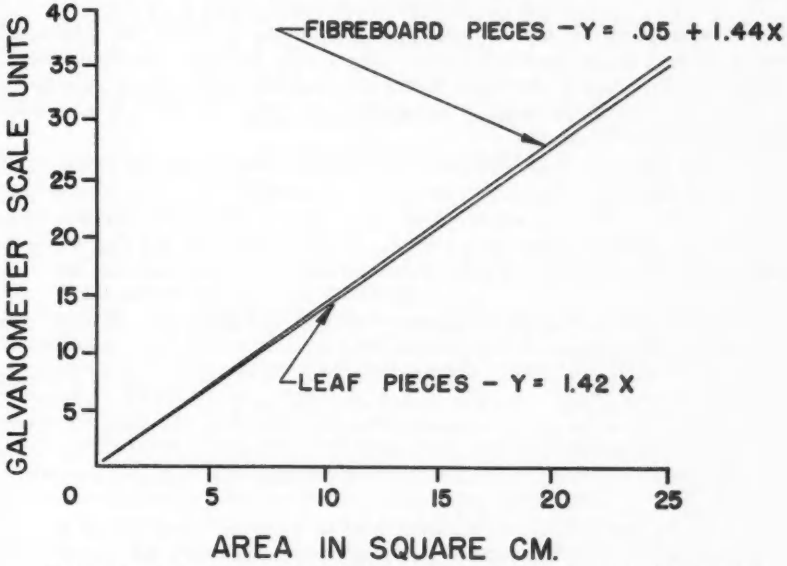

FIGURE 2. A comparison of calibration curves using fibreboard and leaf pieces; curves fitted by the least squares method.

OPERATION

The instruments used with this device are connected as shown in Figure 1. Before the lights are turned on the galvanometer reading is zeroed at 200 on the reversed 200-0 scale. This reversed scale provides a direct relationship between the scale reading and the area of the leaves placed in the light beam.

The lights must be allowed to warm up for 10 minutes. The galvanometer reading is then brought to zero on the reversed scale by adjusting the light intensity through adjustments in the output voltage of the autotransformer. After each measurement when the leaves are cleared from the specimen stage this zero reading should be checked and the light intensity readjusted if necessary. Usually, adjustment at the other end of the scale need only be made every hour or so of operation.

PERFORMANCE

The data in Table 1 show that irradiation of the specimen stage is very uniform. The light intensity in the central part of the beam is slightly higher than that nearer the edges but this difference is not large. In practice the sample of leaves is arranged from the centre of the beam outwards, the result of which is to minimize the error due to position. For samples larger than 20 sq. cm., the percentage error due to position effect would not exceed 1 per cent. For samples smaller than this, a smaller light beam could be used along with a higher light intensity and/or a more sensitive range on the galvanometer.

The upper limit of the optimum sample size is set by the practical consideration of the time required to arrange the leaves in the light beam. With trefoil leaves it has been found that speed of placement in a light beam $5\frac{3}{4}$ inches in diameter usually starts to drop off when the sample size approaches 75 sq. cm.

From Figure 2 it is seen that the differences between the calibration curves for leaf and fibreboard pieces are very small. The difference at the origin is 0.05 scale units while the slope of the curve for leaf pieces is 98.6 per cent of that for fibreboard pieces. These differences are due to such effects as faulty cutting of calibration pieces, errors in scale reading, the slight differences in light intensity at different locations in the beam and possibly a certain amount of uncorrected leaf transparency. Because of problems connected with the cutting and handling of a large number of 1 sq. cm. leaf pieces, it was not practical to compare these curves beyond the 0-25 sq. cm. range. However, this comparison is sufficiently extensive to confirm the similarity of the curves and establish the fact that for practical purposes a complete calibration curve based on fibreboard pieces may be used for the conversion of instrument readings from leaf measurements.

To date the device has only been used in a genetic study of leaf size in birdsfoot trefoil. It has proved to be a very satisfactory instrument for this purpose.

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THE EFFECT OF SWATHING AT DIFFERENT STAGES OF MATURITY UPON THE BUSHEL WEIGHT AND YIELD OF BARLEY[1]

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ABSTRACT

Tests were conducted over a 4-year period with Vantage and Olli barley to determine the earliest stage of maturity at which these grains could be swathed without loss of test weight or yield.

Plots were cut daily over a period of several days using kernel moisture content as a measure of maturity, an attempt being made to keep within the moisture range of 50 to 14.8 per cent. Weights per bushel and yield in bushels per acre were determined at the time of picking up the swath with the combine.

An analysis of variance of the data indicated that Vantage and Olli barley may be swathed at a kernel moisture content of 40 per cent without significant loss of bushel weight or yield. A calculation of the correlation of kernel moisture at swathing with final bushel weight and with yield indicated that an association existed until the 40 per cent moisture level was reached, but the relation was not significant at later stages of maturity.

INTRODUCTION

The accepted prerequisites to harvesting barley by straight combining are uniformity of ripeness and a kernel moisture content of 14.8 per cent or less, for safe storage. The common practices, until recent years, have been to straight combine feed barley and to bind and thresh malting varieties. Recently the swather has come into more general use with both these crops in an attempt to overcome the hazards of the harvest season. The tendency existed, in spite of the advantages of swathing, to allow barley to stand until uniformly mature even though it is subject to loss in quality yield as a result of wind, rain, hail, frosts, or green weeds.

A co-operative study was undertaken at the Experimental Farms at Swift Current, Saskatchewan, and Lacombe, Alberta, to investigate the effect of swathing barley at different stages of maturity. Bushel weight and yield were used as measurements of quality and kernel moisture content employed as the means of determining the stage of maturity. These tests were also studied to establish an optimum time for the swathing operation. The results of the trials conducted during the period 1953 to 1957, inclusive, are reported.

REVIEW OF LITERATURE

Benchley (1) concluded that the weight of the whole barley plant increased steadily until desiccation set in about three weeks before harvest, after which a fall was evident. It was reported by Harlan (4), working

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with Hannchen barley, that the translocation of plant material ceased when the kernel moisture content reached 42 per cent. Later work by Harlan and Pope (5), with Jet barley, indicated that the percentage of water in a growing kernel decreased uniformly day by day until the average of all kernels on the spike was about 42 per cent, at which point the deposition of dry matter was interrupted and the kernels dried very rapidly. Translocation did not become impossible after this stage but continued to a limited extent only. Harlan and Pope (6) further concluded that the immature kernel of Hannchen barley almost certainly abstracted food material from the culm even when severed from the plant. McLean (10) reported that no significant reduction in either yield or weight per 1000 kernels resulted from harvesting O.A.C. 21 a week before maturity. Hewlett and Hewlett (7) obtained satisfactory results regarding quality of barleys harvested by binder and windrower and noted that the grain dried faster in the windrows than when stooked. Schwantes (12), as the result of work done in Minnesota, indicated that the windrower is almost indispensable in weedy fields and that barley cut at 37 per cent moisture dried in the swath 2½ days in advance of standing grain. Rather (11) determined that maximum yields were obtained when grain was cut at a time when the kernels contained approximately 25 per cent moisture, but that moderate losses would occur if left standing until ready to harvest with a combine.

PROCEDURE

Swathing tests were conducted at the Experimental Farm, Swift Current, Saskatchewan, during the harvest seasons 1953 to 1957, excluding 1956. Vantage, a smooth-awned six-rowed feed variety, was grown for these trials. The tests at the Experimental Farm, Lacombe, Alberta, were carried out during the 1954 to 1957 seasons, inclusive, and Olli, a rough-awned six-rowed variety of malting barley, was used. Kernel moisture content at each date of swathing was employed as the measure of the stage of maturity. Previous work by Dodds (3) with wheat defined the practical limits of kernel moisture, for purposes of testing, to be between 50 and 14 per cent. It was assumed that the same limits would be applicable for barley.

A field of barley was divided into four randomized blocks of 20 plots each. Two swaths were cut in each plot, one for moisture determinations and one for yield. Cutting was done with a 14-foot self-propelled swather, the acreage for calculation purposes being one swath wide by 150 feet long at Swift Current and 75 feet long at Lacombe. An exception to this was at Lacombe in 1954 and 1955, when field strips were used and no yield data were obtained. The first swath was cut when the kernel moisture approximated 50 per cent and the operation continued daily thereafter, weather and field conditions permitting, until the grain reached 14.8 per cent moisture or less.

Samples for kernel moisture determinations were collected immediately after cutting, threshed with a laboratory thresher, and cleaned. Moisture determinations for all tests were made on a wet basis by drying 50-gram samples, in triplicate, in a thermostatically controlled electric

TABLE 1.—KERNEL MOISTURE CONTENT, WEIGHT PER MEASURED BUSHEL, AND YIELD DATA, VANTAGE BARLEY 1953, 1954, 1955 AND 1957, SWIFT CURRENT, SASK.

1953				1954				1955				1957 "C"				1957 "S"			
Swathing date	K.M.C. at swathing %	W.M.B. lb.	Yield bu./ac.	Swathing date	K.M.C. at swathing %	W.M.B. lb.	Yield bu./ac.	Swathing date	K.M.C. at swathing %	W.M.B. lb.	Yield bu./ac.	Swathing date	K.M.C. at swathing %	W.M.B. lb.	Yield bu./ac.	Swathing date	K.M.C. at swathing %	W.M.B. lb.	Yield bu./ac.
July 30	55.9	44.3	28.4	Aug. 23	33.8	47.0	60.7	Aug. 8	44.8	50.3	45.8	July 25	51.4	41.5	36.8	July 29	46.4	44.6	38.4
July 31	56.7	43.9	27.8	Aug. 24	35.6	47.4	56.8	Aug. 9	42.8	50.8	52.7	July 26	49.6	42.1	35.6	July 30	44.1	43.9	40.0
Aug. 1	57.5	44.0	31.2	Aug. 30	19.8	45.3	58.7	Aug. 10	40.0	50.1	47.8	July 27	49.7	43.6	40.2	Aug. 2	43.2	44.6	40.8
Aug. 3	52.2	46.0	38.4	Aug. 31	17.5	46.4	58.5	Aug. 11	40.2	51.1	54.9	July 29	45.9	44.2	44.5	Aug. 3	44.6	45.0	38.9
Aug. 4	50.4	46.9	36.8	Sept. 1	18.9	45.1	55.6	Aug. 12	36.2	51.3	53.6	July 30	44.8	44.4	40.9	Aug. 5	36.8	43.8	38.1
Aug. 5	48.5	47.3	38.7	Sept. 2	19.2	45.8	48.9	Aug. 15	33.0	51.9	54.6	July 31	43.6	46.2	45.5	Aug. 7	32.2	45.6	41.1
Aug. 6	46.0	47.3	42.0	Sept. 3	17.2	45.1	51.3	Aug. 16	27.4	51.3	53.1	Aug. 1	41.6	46.9	45.4	Aug. 8	30.0	45.8	40.0
Aug. 7	45.4	48.8	44.0	Sept. 4	19.8	45.1	43.3	Aug. 17	23.7	53.0	57.9	Aug. 2	39.9	45.0	49.3	Aug. 9	22.5	45.0	43.0
Aug. 8	41.7	47.1						Aug. 18	25.6	49.0	56.9	Aug. 3	43.6	46.6	43.8	Aug. 12	21.0	44.5	42.2
Aug. 10	36.4	48.6						Aug. 19	20.2	48.5	60.3	Aug. 5	25.2	46.8	48.2	Aug. 13	14.8	44.4	41.1
Aug. 11	36.4	47.5						Aug. 20	12.2	48.5	61.9	Aug. 7	30.8	46.6	42.8	Aug. 14	15.0	44.8	44.3
Aug. 12	28.7	47.8										Aug. 8	26.7	46.5	43.0	Aug. 15	15.2	45.4	42.8
Aug. 13	23.8	46.4										Aug. 9	21.5	47.0	46.3	Aug. 16	12.8	44.2	43.9
Aug. 14	18.7	48.3										Aug. 12	18.9	46.1	50.4				
Aug. 15	17.3	47.3										Aug. 13	18.6	46.5	50.2				
Aug. 17	17.3	48.1										Aug. 14	15.2	45.5	50.6				
Aug. 18	12.7	48.3										Aug. 15	15.1	46.8	50.1				
Aug. 19	17.1	49.3										Aug. 16	12.8	46.6	49.5				
Aug. 20	13.0	48.6																	
Aug. 21	12.8	47.8																	
L.S.D. P = .05	2.52	1.02			3.74	1.67	10.01		5.60	1.90	5.40		6.28	2.44	8.44		5.71	N.S.	N.S.
S.E.M. %	2.58	0.76			8.08	1.24	6.35		6.21	1.30	3.43		6.72	1.98	6.59		7.47	2.07	6.54

oven for 20 hours at 98°C. at both locations. A Halross electric moisture tester was used at Swift Current for determinations when the kernel moisture fell below 27 per cent.

The rate of drying in the swath was determined and recorded from samples of grain collected daily from each swath. Observations were made on quality of swath, as swathing progressed, from the standpoint of stubble support.

The swaths were recovered with a combine and threshed when the kernel moisture content reached 14.8 per cent or less. The recorded weight of grain from each plot was used to calculate yield in bushels per acre on the basis of a 48-lb. bushel. The actual weight per measured bushel was determined at the same time on the individual plots at Swift Current. At Lacombe, weight per measured bushel was calculated on bulked samples for each date.

RESULTS AND DISCUSSION

Vantage Barley at Swift Current

The results of the experimental work at Swift Current with Vantage barley for the period under consideration are shown in Table 1.

The very early swathing dates in 1953 and 1957 indicate that weight per measured bushel tends to increase to some maximum point and then to level off or decline, depending on weather conditions during the harvest period. This appeared to take place in 1953 at a fairly early stage and fluctuated daily from the period of 45.4 per cent kernel moisture until the last day of cutting. Wet weather and poor field conditions in 1954 delayed harvest operations until the kernel moisture content reached 33.8 per cent. The results indicated that periodic wetting of the standing grain by rain in the evenings and drying in the warm sun during the day had an adverse effect on kernel weight. LeClerc and Breazeale (9), in their study of the effect of rain and dew on grain, reported that this was due to a leaching of plant material, while Bracken and Bailey (2), and Kiesselback and Lyness (8) also noted a loss of test weight of wheat following periods of wetting and drying. The grain swathed, in 1955, at a kernel moisture content of 23.7 per cent had the highest weight per measured bushel when recovered with the combine. This was not significantly higher than the final bushel weight of that cut at 40.2 per cent kernel moisture. Grain swathed when the moisture content was less than 23.7 or more than 40.2 per cent resulted in significantly lower final bushel weights. Two fields of barley were used for the 1957 tests. The highest final bushel weight in Field "C" was recorded from the sample swathed at 21.5 per cent moisture. The test showed that weight per measured bushel did not vary significantly after the grain developed to the stage of maturity reached at 45.9 per cent kernel moisture. The data from Field "S" showed no significant difference for final bushel weight through the entire range of the test.

Complete yield results for 1953 were not obtained because of damage to the swaths by a very severe wind. The information, however, did indicate an upward trend as maturity advanced, such a trend being better

illustrated by the 1955 and 1957 data. The highest yield in 1954 was obtained at the first date of swathing due to the lateness of the harvest season that year. It was apparent that the lack of earlier data prevented reasonable conclusions from being drawn. The effect of adverse weather conditions, during this critical harvest period, are reflected by a decrease in yield due to shattering. The maximum yield in 1955 was noted at the last swathing test. An examination of the trend in yields from the 40.2 per cent moisture level onwards might suggest that this could be due to chance fluctuations. It was not, however, significantly higher than that recorded 3 days earlier, and only slightly higher than the yield resulting from swathing at the 40.2 per cent kernel moisture content level. The 1957 results, from Field "C", showed that the highest yield was found in the sample of grain combined from the test swathed at 15.2 per cent moisture. This was not significantly higher than the threshed samples of the test cut at 45.9 per cent moisture. The data from Field "S" showed no significant difference in yield for the entire test.

The degree of association between kernel moisture content at swathing and final bushel weight was determined by the calculation of correlation coefficients, using means of these characters. A similar computation was also made between kernel moisture content at swathing and yield. These are presented in Table 2. The complete data for any one year were considered first. A second calculation from the initial swathing date to approximately the 40 per cent kernel moisture stage was then made, with a third calculation from 40 per cent to the end of the test.

Kernel moisture and bushel weight were significantly and negatively correlated for the full range of the data in all tests except 1955. This association remained quite firm in the moisture range down to 40 per cent, but no significant relationship appeared from that point to the end of the test in any one year.

TABLE 2.—TABLE OF CORRELATION COEFFICIENTS, VANTAGE BARLEY, SWIFT CURRENT, SASK.

Year	Range of kernel moisture content %	Number of tests	r Between kernel moisture and bushel weight	r Between kernel moisture and yield
1953	55.9 — 12.8	20	−.698**	—
	55.9 — 41.7	9	−.886**	—
	55.9 — 45.4	8	—	−.946**
	36.4 — 12.8	11	−.199	—
1954	33.8 — 19.8	8	+.843**	+.422
1955	44.8 — 12.2	11	+.374	−.867**
	44.8 — 40.2	4	−.183	−.495
	36.2 — 12.2	7	+.611	−.903**
1957 "C"	51.4 — 12.8	18	−.678**	−.797**
	51.4 — 41.6	7	−.951	−.866*
	39.9 — 12.8	11	−.233	−.590

* Significant at P = .05
** Significant at P = .01

TABLE 3.—KERNEL MOISTURE CONTENT, WEIGHT PER MEASURED BUSHEL, AND YIELD DATA, OLLI BARLEY 1954, 1955, 1956 AND 1957, LACOMBE, ALTA.

1954			1955			1956				1957			
Swathing date	K.M.C. at swathing %	W.M.B. lb.	Swathing date	K.M.C. at swathing %	W.M.B. lb.	Swathing date	K.M.C. at swathing %	W.M.B. lb.	Yield bu./acre	Swathing date	K.M.C. at swathing %	W.M.B. lb.	Yield, bu./acre
Sept. 2	52.2	40	July 30	58.0	41	Aug. 8	56.8	41	40.7	July 28	51.0	44	28.1
Sept. 7	51.8	40	Aug. 1	53.4	42	Aug. 10	52.8	41	44.2	July 29	48.4	46	31.9
Sept. 8	53.0	44	Aug. 2	52.8	44	Aug. 11	50.6	41	47.7	July 30	47.2	46	36.4
Sept. 10	49.8	43	Aug. 3	50.8	45	Aug. 12	47.4	44	48.1	July 31	47.6	47	32.6
Sept. 13	42.4	45	Aug. 5	45.0	46	Aug. 13	48.1	42	57.6	Aug. 3	42.4	49	39.0
Sept. 20	31.6	45	Aug. 9	42.8	46	Aug. 14	46.1	42	65.0	Aug. 4	39.0	47	35.6
Sept. 21	29.8	46	Aug. 10	39.4	46	Aug. 15	40.4	44	63.7	Aug. 5	34.6	45	33.9
Sept. 22	21.8	47	Aug. 11	29.2	47	Aug. 18	39.9	45	66.3	Aug. 6	30.1	48	35.9
Sept. 23	18.0	45	Aug. 12	24.2	47	Aug. 20	26.0	45	58.9	Aug. 7	27.1	46	37.0
Sept. 24	12.4	48	Aug. 13	24.8	47	Aug. 21	23.8	45	58.1	Aug. 8	26.4	48	38.5
Sept. 25	15.0	47	Aug. 15	14.4	48	Aug. 22	21.1	42	60.7	Aug. 9	21.4	47	36.2
Sept. 30	16.0	46				Aug. 23	20.0	47	62.8	Aug. 12	19.2	47	36.6
										Aug. 13	18.3	48	36.5
L.S.D. (P = .05)							5.5		6.7		10.0		6.4
S.E.M. %							4.49		4.42		8.96		5.83

A somewhat similar pattern was shown when kernel moisture content and yield were correlated. These factors showed a significant relationship for the full range of the data in 1955 and 1957. A firm negative correlation down to 40 per cent moisture was shown in 1953 and 1957. The significant correlation coefficient, in the lower moisture ranges, in 1955 may be attributed to a continuing effect of kernel moisture affected by a very favourable harvesting period during which time the weather was hot and dry and was not accompanied by wind, rain, or dews. This apparent gain was more than outweighed by the deterioration due to the hazards normally encountered during other harvest seasons. The analysis of variance for the data from Field "S" in 1957 indicated no significant difference in either bushel weight or yield, so no further comparisons were necessary.

Olli Barley at Lacombe

The results of experimental work with Olli barley at Lacombe for the years 1954 to 1957, inclusive, are shown in Table 3.

Test weights shown were from bulked samples for each date of swathing and therefore show trends only. The weight per measured bushel tended to increase toward a maximum and then level off in all tests. The maximum bushel weight for 1957 was obtained from a sample of the threshed grain that was swathed at 42.4 per cent kernel moisture. A levelling-off or slight decline was noted from that stage until maturity. The maximum weight for other years was recorded from tests swathed at somewhat lower moisture contents.

Yield results were available for only the last 2 years of this test and significant increases were obtained in both years as the moisture content of the swathed sample decreased to about the 40 per cent level. Thereafter a slight decrease in yield in 1956 and a levelling-off in 1957 was noted.

TABLE 4.—TABLE OF CORRELATIONS COEFFICIENTS OLLI BARLEY, LACOMBE, ALTA.

Year	Range of kernel moisture content %	Number of tests	*r* Between kernel moisture and bushel weight	*r* Between kernel moisture and yield
1954	52.2 — 16.0	12	−.836**	—
	52.2 — 42.4	5	−.428	—
	31.6 — 16.0	7	−.576	—
1955	58.0 — 14.4	11	−.868**	—
	58.0 — 42.8	6	−.910*	—
	39.4 — 14.4	5	−.976**	—
1956	56.8 — 20.0	12	−.457	−.616*
	56.8 — 40.4	7	−.790*	−.864*
	39.9 — 20.0	5	+.052	+.666
1957	51.0 — 18.3	13	−.414	−.592*
	51.0 — 42.4	5	−.978**	−.950*
	39.0 — 18.3	8	−.400	−.473

* Significant at P = .05
** Significant at P = .01

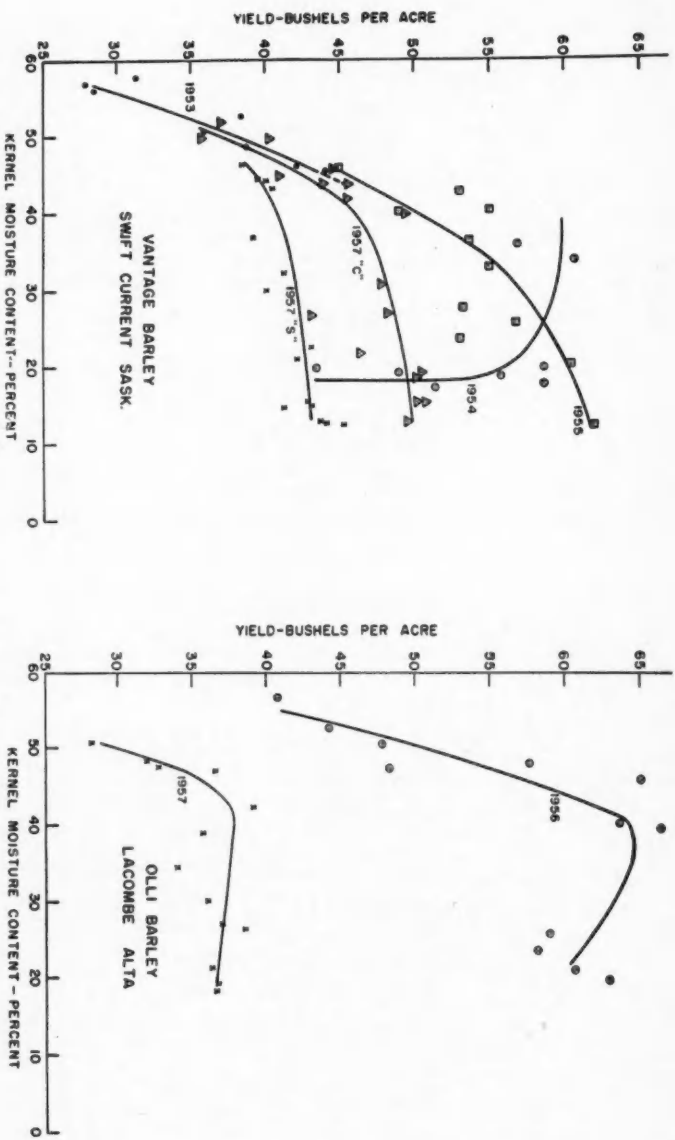

FIGURE 1. Kernel moisture content of barley swathed at different stages of maturity related to yield.

The yield in 1956 increased sharply as the kernel moisture of the swathed sample decreased to about the 40 per cent level. Thereafter it declined, largely due to swather losses in a heavy and badly lodged crop of Olli barley grown on fallow. The data for 1957 indicated that the minimum yield was obtained from a sample that was swathed at a kernel moisture content slightly in excess of 40 per cent and then remained fairly constant for the remainder of the test. This crop was grown on stubble and stood up well throughout the entire swathing period.

Correlation coefficients calculated from the data obtained at Lacombe are presented in Table 4. Kernel moisture content at swathing and bushel weight were significantly and negatively correlated in 1954 and 1955 for the data covering the range of the test, but this association was not apparent in 1956 and 1957. The correlation coefficient in the range from the beginning of swathing until the 40 per cent level was significant in all years except 1954, and was highly significant in 1957. The correlation below 40 per cent kernel moisture was not significant in any year except 1955. This would indicate that bushel weight increased with maturity until approximately the 40 per cent moisture level was reached, at which stage the association became inconsistent.

The correlation coefficient for moisture and yield was significant and negative for the full range in both years when yield was obtained. A significant negative correlation also existed for the range down to 40 per cent, while below this range no significant relationship existed. The positive relationship in this range in 1956 reflects the downward trend in yield as a result of swather losses in that year.

The trend of yield when related to kernel moisture content at swathing is presented in Figure 1. The levelling out of the yield of Vantage barley is apparent at the 40 per cent kernel moisture stage for 1955 and 1957, and the losses from shattering in 1954 are well illustrated. A distinct peak yield at the 40 per cent kernel moisture level is noted from the results of the tests on Olli barley for both years.

Quality of Swath and Rate of Drying

The swaths were well formed and adequately supported on the stubble where suitable stubble was available. The extra weight of green grain did not cause the swath to fall to the ground any more than a swath of mature grain placed on stubble of similar height.

It was established, from data collected on the rate of drying in the swath, that barley cut at 40 per cent kernel moisture content could be recovered with a combine 4 days later if suitable weather conditions prevailed during that period. This stage of maturity, which occurred 9 to 11 days in advance of the conditions normally accepted for straight combining in the Swift Current area, means that the 5- to 7-day advantage could be gained. A similar pattern occurred at Lacombe but frequent showers often obscured the advantage. Swaths cut at a lower moisture content dried in a slightly shorter period, being 3 days when cut at 25 per cent kernel moisture.

CONCLUSIONS

1. Tests with Vantage and Olli barley have indicated that these crops may be swathed at a stage of maturity indicated by a kernel moisture content of 40 per cent. An analysis of the results reveals that neither bushel weight nor yield are significantly reduced by cutting at this stage as compared to cutting at a later stage.

2. Harvest operations may be advanced, by swathing, as much as 5 to 7 days ahead of a stage of maturity accepted as suitable for straight combining.

3. The swath is well formed at this stage and remains supported on the stubble, thus reducing losses from rain, hail, and shattering, and avoiding some of the mechanical losses associated with straight combining.

4. The swath may be recovered with a combine during normal harvesting weather 4 days after swathing.

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NOTE ON NORLEA PERENNIAL RYE GRASS

This newly licensed variety was developed at the Forage Crops Division, Central Experimental Farm, Ottawa, Ontario. Its significant attribute is that it possesses sufficient hardiness to survive and be productive in areas where, heretofore, the species failed to overwinter satisfactorily and therefore was of no value.

SOURCE MATERIAL AND BREEDING METHODS

The original material from which this variety evolved comprised some 100 introductions from many parts of the world. In 1940, a spaced plant nursery of approximately 15,000 plants, representative of the introduced lots, was established. From the outset emphasis was placed on winter survival. By repeated selection and progeny evaluation through six generations the present measure of hardiness has been reached. At the fourth generation stage of development for winter hardiness the material being carried forward was the progeny of twelve desirable clons. Selection then continued in these progenies for two more generations. By this means the hardiness of the variety was established at a reasonably satisfactory level, while a definite improvement was brought about in other agronomic characteristics.

VARIETAL CHARACTERISTICS

The variety does not deviate in botanical characteristics from other varieties of the species.

AGRONOMIC CHARACTERISTICS

Plant type—Leafy, somewhat later in maturity than short-ley rye types.

Winter hardiness—Considered to be more winter hardy than other known varieties. At Ottawa and other locations this variety has survived and given substantial yields when other varieties of European and Pacific origin have been almost completely winter killed.

Disease resistance—Susceptible to leaf rust in some areas. This susceptibility does not appear to affect yield.

Forage yield—Possibly because of superior hardiness Norlea has in most tests outyielded other varieties.

Seed yield—A heavy seed yielder. It is considered the equal of other rye grasses in this regard. Because of winterkilling in other varieties comparative data are not available.

PROBABLE USES

This variety should have a place in permanent and irrigated pasture production and is a good possibility as a desirable, persistent and cheap turf species.

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Experimental Farms Service,
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March 26, 1958

NOTE ON THE LOCATION OF GENES FOR DWARFING IN REDMAN WHEAT[1]

The spring wheat variety, Kenya Farmer, has been used extensively in crosses for its rust resistance, and several combinations have produced dwarfs in the F_1 (2). In the present study, dwarf F_1 plants resulted from crosses between the 21 Redman monosomic lines and Kenya Farmer. Segregation for dwarf and normal growth habit occurred only with lines VIII and XIII (Figure 1), the remaining 19 lines producing all dwarf plants. Of the six F_1 plants grown from each of the segregating lines, four were normal in growth and two were dwarf from line VIII, while from line XIII there were three normals and three dwarfs. All plants in these two lines were analysed cytologically to determine their chromosome numbers. Chromosome counts of dwarf plants were made on root tips and in all cases they proved to have 42 chromosomes (Figure 2). Analysis of pollen mother cells of the plants with normal growth revealed that they were monosomic (Figure 3). Analysis of root tips of several plants in the other F_1 lines showed that both monosomic and disomic plants were present.

From this preliminary investigation it is concluded that at least three complementary dominant genes condition dwarfing in this cross. The absence of any one of these genes allows normal plant growth. The data indicate that Redman possesses two genes for dwarfing located on chromosomes VIII and XIII, respectively. The other complementary and non-allelic gene (or genes) must be carried by Kenya Farmer since dwarfing is only expressed in hybrids of the two varieties, and not in either variety itself. As summarized by Morrison (2), many previous workers have found that dwarfing in wheat is governed by complementary factors.

It is interesting to note that genes for stem rust resistance have been located on chromosomes VIII and XIII in Redman (1) and as a consequence there is a possible linkage between these genes and the genes for dwarfing. Perhaps a similar situation exists in some other rust-resistant varieties.

ACKNOWLEDGEMENT

The authors are indebted to W. E. Clark, Canada Department of Agriculture Research Laboratory, for preparation of the photographic plate.

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May 22, 1958

[1] Contribution No. 234 of the Cereal Crops Division, Experimental Farms Service, Canada Department of Agriculture, and Contribution No. 6 of the Canada Department of Agriculture Research Laboratory, Winnipeg, Manitoba.

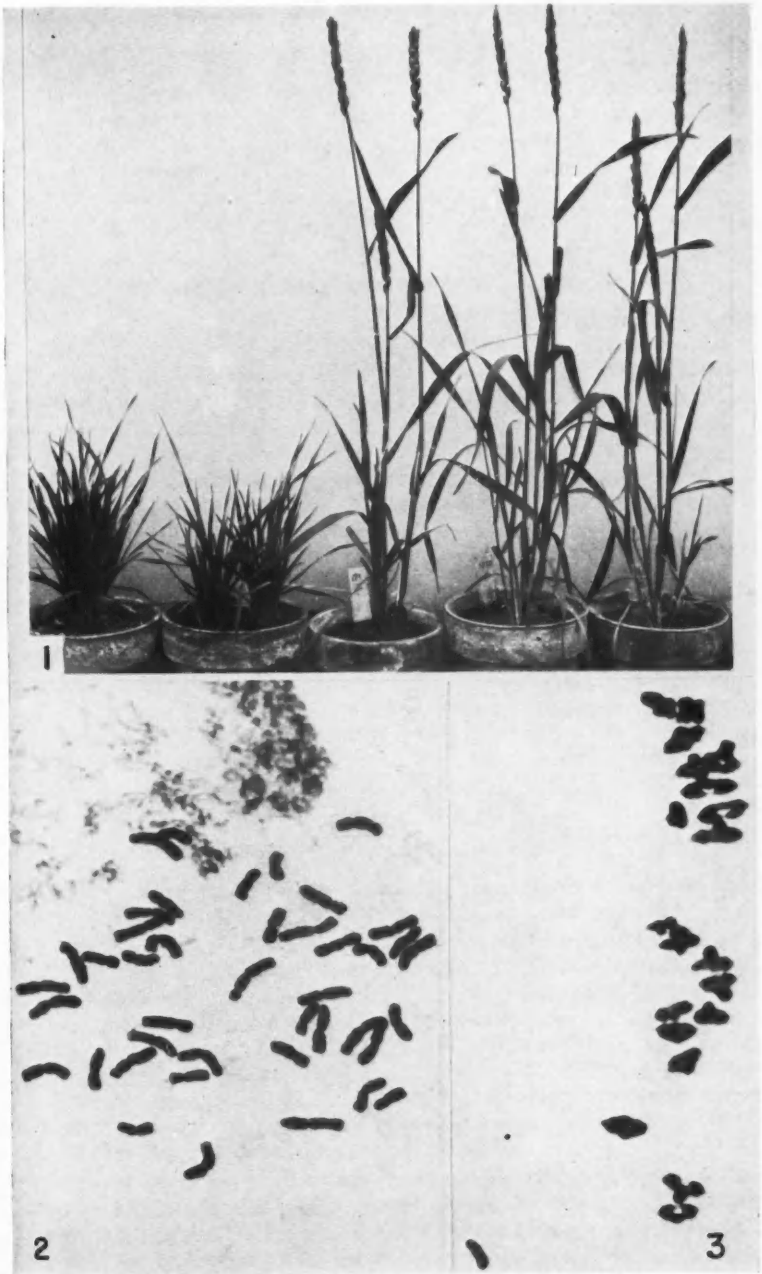

FIGURE 1. Dwarf and normal F_1 plants from the cross Redman monosomic XIII × Kenya Farmer.

FIGURE 2. Root tip cell with 42 chromosomes (disomic) from a dwarf plant shown in Figure 1.

FIGURE 3. Pollen mother cell with $20^{II}1^{I}$ (monosomic) from a plant with normal growth shown in Figure 1.

NOTE ON CUMINO SWEET CLOVER

This newly licensed variety was developed at the Dominion Forage Crops Laboratory, Saskatoon, Saskatchewan. Its outstanding characteristic is that it is coumarin free. Coumarin in ordinary sweet clover is responsible directly for 'Melilot taint' and indirectly for losses of livestock from sweet clover disease. This new variety should reduce or eliminate these undesirable characteristics of the crop.

SOURCE MATERIAL AND BREEDING METHODS

The coumarin-deficient gene was obtained in a few seeds received from W. K. Smith, University of Wisconsin, in 1946. Dr. Smith obtained his material from an interspecific cross between *M. alba* and *M. dentata*, backcrossed to *M. alba*. At Saskatoon, during the course of development, these original introductions were backcrossed to selected *M. alba* plants five times. The progenies resulting from each backcross were selfed and the selfed progenies were screened for the coumarin-deficient character and desirable agronomic type. One particularly promising coumarin-deficient line resulted and this line was crossed with three plants from Arctic and one from Pioneer. Several F_1 plants were grown and coumarin-deficient plants were selected from the F_2 progenies. There were 71 plants from the three Arctic parents and 17 from the Pioneer. These 87 plants were increased by using honey bees under cage isolation to produce Syn. 1 seed. From this seed a field plot, isolated more than a mile from any other sweet clover, was established. This isolation consisted of 2 acres of plants, spaced 3 feet in each direction, and produced the stock that is now being offered as breeders' seed. Each plant in this isolation was tested for coumarin content with negative results. Approximately 600 pounds of seed were harvested.

VARIETAL CHARACTERISTICS

(1) *Flower Colour*—Cumino has white flowers that are indistinguishable from other *M. alba* plants of coumarin-containing varieties.

(2) *Coumarin Status*—No coumarin is present when NaOH is used as the extraction agency. When individual plants are tested, using a photo-fluorometer, a few give readings of .001 to .004 per cent coumarin. This occurs in a relatively small proportion of the population and is not considered significant, since plants of Arctic and Erector regularly show .20 to 1.00 per cent coumarin with the same test. There are no characteristics that readily distinguish the new variety from other varieties of white-flowering sweet clover of the Arctic type, except, of course, the lack of coumarin.

(3) *Seasonal Growth*—In the first year the plants are somewhat more upright than Arctic. Second-year plants are medium to tall and resemble Arctic in shape and size. Certain plants are bushy and leafed to the base, but no dwarf types have been observed. Spring growth in the second year starts at about the same time as Arctic.

(4) *Winter Hardiness*—The variety was observed following the winter of 1956-57, and proved equivalent in hardiness to Arctic and Erector. In previous generations it has been compared to Erector and found equal to that variety.

(5) *Disease Resistance*—Ratings for disease in the breeding nurseries have compared favourably with those for Arctic and Erector.

(6) *Maturity*—The maturity date is about the same as for Arctic and from 7 to 10 days later than Erector. At Saskatoon it flowers from June 28 to July 7, and is ready to harvest for seed between August 20 and September 1.

(7) *Shattering*—The seed does not shatter as readily as in the variety Erector, but shatters to about the same degree as Arctic.

(8) *Forage Yield*—Comparative yield tests have not yet been possible on a replicated-plot basis. However, the variety Erector has been grown as a check in nurseries throughout the breeding program (Erector does not cross with *M. alba* varieties). In all years the average vigour and yield scores have been equal or better than those for Erector.

(9) *Seed Yield*—As with forage yield, the variety has been compared throughout to Erector. On the basis of seed scores and actual plant yields it can be expected to equal Erector or Arctic.

DISCUSSION

When Cumino seed is available in larger quantities it is likely to replace most of the Erector and Arctic sweet clover now used for hay, silage and pasture. Since coumarin in plant material can be detected accurately by the use of a simple chemical test, and since the presence of coumarin is controlled by a single dominant gene, it will be possible to keep a strict check on the purity of this variety.

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March 26, 1958

NOTE ON A PORTABLE CUTTING BOX FOR FORAGE CROP SAMPLES[1]

Lack of a satisfactory cutting box for chopping forage crop samples for dry matter determination and chemical analyses has been a problem in areas with trials located at long distances from central research institutions.

A portable forage crop cutting box was devised which was efficient, compact, and easy to transport between various test areas, and is herein described.

This machine consists of a small clover and chaff cutter of the manually-operated, two-knife, bench type mounted on an angle iron frame and powered by a fractional h.p. gasoline engine. (*See Figure 1*). The cutting box is the Bentall C.P.M. Clover and Chaff cutter[2]. It has a 5-inch throat, with spring tension, positive drive feed rolls. Length of cut is theoretically $\frac{1}{4}$-inch and the capacity of this chopper is from 1 to 2 pounds of green material per minute.

The assembled machine is 36 inches long, 26 inches wide and 40 inches high to the top of the fly-wheel cover. Its total weight is 195 pounds. The cost, excluding labour, was $125.00.

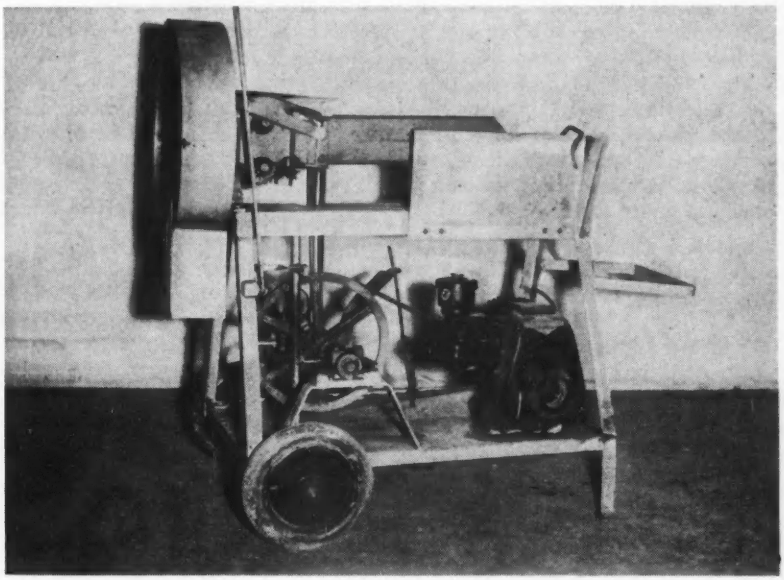

FIGURE 1. Power unit, clutch assembly, Bentall clover cutter and frame construction.

[1] Contribution No. 108 from the Illustration Stations Division, Experimental Farms Service, Canada Department of Agriculture, Ottawa, Ont.

[2] E. H. Bentall Co. Ltd., Heybridge, Maldon, England.

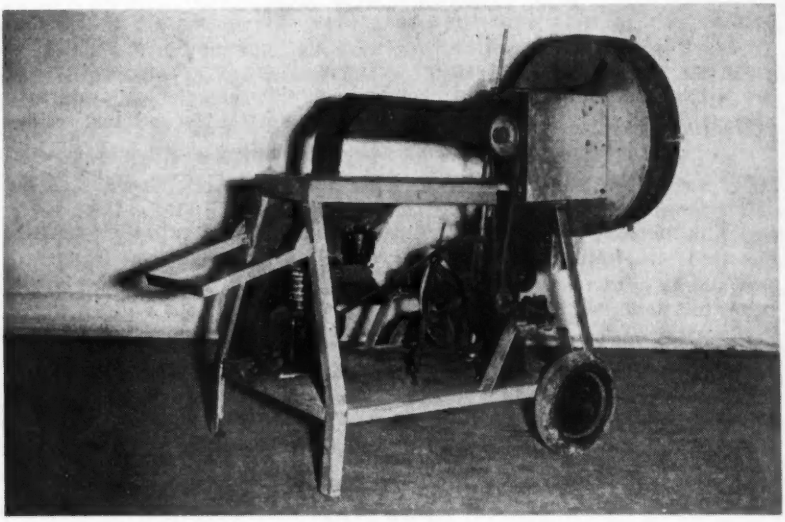

FIGURE 2. Jack shaft mounting for speed reduction, belt tightener and power transmission assembly. Note ground wheels to facilitate moving in plot area.

The machine is V-belt driven through a Jack shaft to reduce the speed of the motor. A sprocket clutch disengages the power. The fly-wheel cover can be opened for cleaning after each sample. Ten-inch wheels are placed under one end of the machine (*Figure 2*), to enable it to be moved easily in a plot area.

ACKNOWLEDGEMENT

Acknowledgement is made to the Engineering Section of the Field Husbandry, Soils and Agricultural Engineering Division, for developing the author's ideas and assembling this machine.

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June 12, 1958

NOTE ON A FLOTATION APPARATUS FOR REMOVING INSECTS FROM SOIL[1]

ABSTRACT

In studies on the life histories and habits of root maggots and their predators in Prince Edward Island, a method was required by which larvae and puparia could be separated uninjured from soil. The flotation methods described recently by Lafrance (1) and Read (2) for collecting puparia were unsuitable because of the stirring or pressure mechanisms, which often injured the insects. A new apparatus lacking these mechanisms was developed and provided a rapid and efficient method for separating many kinds of insects from sandy, loam, or clay loam soils.

DESCRIPTION OF APPARATUS

The apparatus consists of a large water tank, a washing cylinder, a protective hood through which a garden hose fitted with a common-type spray nozzle is inserted (Figures 1-3), and a galvanized iron tray, and screening materials that are used to transfer and pick up the insects from the debris in the cylinder and the tray.

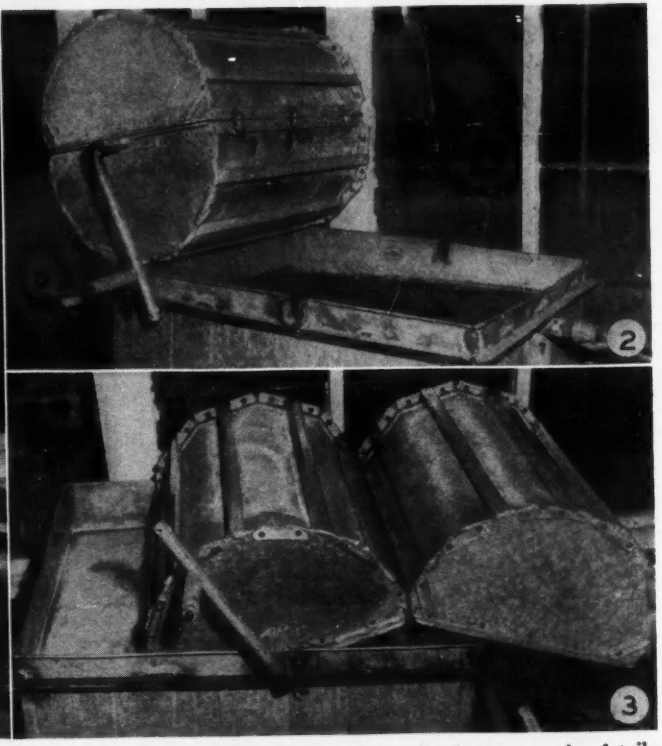
The tank and tray are made of heavy-gauge galvanized steel, the tank being 30 x 27 x 48 inches and the tray 30 x 20 x 6 inches. The upper part of the tank is reinforced with 1-x-1-x-$\frac{1}{4}$-inch angle iron welded around the outside 2 inches from the top. To support the axle of the cylinder, pieces of 1-x-$\frac{1}{4}$-inch flat iron bent into a U shape are welded to the angle iron 12 inches from one end of the tank and the portion of the tank enclosed by the U-shaped flat iron is cut out (Figure 2). The end of the tank closer to the axle slots is fitted with a 1-inch overflow pipe connected $2\frac{1}{2}$ inches from the top and a 2-inch drain-pipe connected about 6 inches from the bottom. The opening from the tank into the drain-pipe is covered with 8-mesh screening.

The frame of the washing cylinder (Figures 2,3) is of angle aluminum with 16-mesh aluminum screening around the inside and aluminum or galvanized-iron sheeting on the ends. The cylinder is 26 inches long by 18 inches in diameter and is divided longitudinally into two halves. The frames of the ends and base of each half are made of 1-x-1-x-$\frac{1}{4}$-inch angle aluminum and the longitudinal strips of 1-x-1-x-$\frac{1}{8}$-inch angle aluminum, all joints being welded. The axle is of 1-inch aluminum rod, 3-inch lengths being welded to the centre of each end of the cylinder. The crank is of 1-inch tubular aluminum welded to one end of the axle. The screening is fastened to the inside of the cylinder with $\frac{3}{4}$-inch quarter-round pine connected to the ends and base of the frame with $\frac{3}{4}$-inch brass screws, and the sheeting is fastened to the semicircular ends of the frame with $\frac{3}{4}$-x-$\frac{1}{8}$-inch brass bolts.

The hood (Figure 1) prevents water from splashing from the tank during the washing operation. It is of canvas and transparent celluloid supported by a framework of $\frac{3}{4}$-inch dressed pine 22 inches in height. The base of the frame rests on the angle-iron bracing that surrounds the tank.

[1] Contribution No. 3831, Entomology Division, Science Service, Canada Department of Agriculture, Ottawa, Ont.

FIGURE 1. Soil-washing apparatus in operation.



FIGURES 2, 3. Washing cylinder and top of galvanized-steel tank, showing construction details.

METHOD OF OPERATION

The apparatus may be used to process soil taken directly from root-crop, sod, or stubble fields but clumps of sod should first be pulled apart by hand.

About 1 cubic foot of soil is washed at a time. The tank is filled with water to the level of the overflow pipe and the soil sample is placed in the cylinder. The spray nozzle is then turned on and the cylinder is revolved about 20 times, or until the soil and other fine material have passed out of the cylinder and settled to the bottom of the tank. When the washing is completed, the cylinder is opened and a piece of 16-mesh screening is used to transfer the material floating on the water to the galvanized-steel tray, which is filled to a depth of about 5 inches with water. Here, the insects may be picked off with a small, spoon-shaped strainer fitted with a short handle. To collect the insects that do not rise to the surface of the water, the cylinder is removed from the tank and the half that contains the insects and coarse debris is dipped several times in and out of the tray, in clear water. The insects rise to the surface of the debris in the cylinder and may be picked out with forceps.

When all the insects are collected, the cylinder is returned to the tank and inverted as shown in Figure 3 to empty out the gravel and coarse debris. The procedure is then repeated with another cubic foot of soil. When the quantity of soil in the tank prevents the cylinder from revolving freely, the drain-pipe is opened to allow the water to drain from the tank and the soil and other debris are discarded.

DISCUSSION

The apparatus may be used for collecting many types of insects from soil. The washing operation is carried out in a closed system and all insects larger than the openings in the screening, about $1\frac{1}{2}$ mm., may be collected. The most important advantage over other types of separating devices is that no stirring or other pressure mechanism that might injure the insects is involved. The water cushions contact between the insects and the sides of the cylinder. When the cylinder is revolved clockwise, the longitudinal strips of angle aluminum, acting as scoops, help to accelerate the washing by forcing water into the cylinder.

A cubic foot of soil can be processed in less than 5 minutes. The time required to pick the insects from the debris remaining in the cylinder depends on the amount of organic material and the number of insects in the sample; in work with root maggots, more than 1000 puparia were separated in about 6 hours from soil collected in the field. In tests with sandy, loam, and clay loam soils with known numbers of different species of root maggot larvae and puparia, and of coleopterous larvae and adults, 100 per cent of the insect specimens were recovered uninjured.

ACKNOWLEDGEMENTS

The author wishes to thank C. P. Duffy, Assistant Technician, Charlottetown, Prince Edward Island, for assistance in constructing the apparatus. Grateful acknowledgement is also made to the firm of Proud and Moreside, Charlottetown, for use of their facilities and equipment.

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July 9, 1958

NOTE ON THE VEGETATIVE REPRODUCTION OF PEACH CUTTINGS[1]

Seedling rootstocks, mainly derived from "pits" of the Lovell peach from drying yards in California, are used in propagating the peach *Prunus persica. L.* in the eastern United States and Canada. Lovell seedlings appear remarkably uniform in vigour and appearance, but typical seedling variability is evident in the existence of mildew-susceptible eglandular forms (4) and in the performance (2) of varieties budded on Lovell in replant orchards where nematodes, toxins and aphids could be contributing factors. Such variability complicates the interpretation of results from seedling roostock experiments and could be controlled by vegetative propagation from a single clone if practicable. Some success in obtaining rooted peach cuttings has been achieved with mist propagation by several workers (1) (3) (5), but considerable difficulty is still encountered locally in establishing the rooted cutting in an outdoor environment where it may be budded or grown to orchard size.

Numerous unsuccessful efforts at Harrow to propagate the peach led to the installation of a small air-conditioned mist tunnel, according to directions given by Sweet and Carlson (5). This unit maintained excellent turgidity and induced rooting within 3 weeks in firm softwood peach cuttings that were struck in a sand or perlite[2] medium after wounding and dipping in 1000 p.p.m. indolebutyric acid 50 per cent alcohol solution for 2 seconds. Such cuttings, however, suffered considerable root damage on removal from the medium for examination prior to transfer to a shaded greenhouse soil because the roots are thick, brittle and devoid of root hairs.

This type of loss was eliminated by following the suggestion of J. Scatterty, Head Gardener, who advised the use of 2¼" peat containers[3] containing perlite for each cutting. The individual containers permitted examination without damage since rapidly developing roots penetrated them readily. At the end of the "mist" period the containers and cuttings were plunged into a 4-inch clay pot which was filled with a greenhouse compost soil high in organic matter. The cuttings, placed under heavy shade and watered twice daily, began to make shoot growth and in 2 weeks were transplanted into the outdoor nursery where temporary shading was found advisable (Figure 1).

Firm softwood cuttings from juvenile nursery-grown Yunnan 1-year peach seedlings taken on May 30, 1957, and handled in the manner described, were transplanted into the nursery on June 28 and attained an average height of 15 inches before losing their leaves in November. Over-winter survival of the cuttings appears to be satisfactory and "budding" will be done in July, 1958. Cuttings of other varieties and seedlings struck later suffered somewhat from lack of shade when transplanted into the nursery.

Considerable effort and expense are involved in the procedure described here which might limit its usefulness in the commercial propagation of peach varieties. This phase of the problem is now being investigated as there are no reports on the orchard performance of own-rooted peach trees.

[1] Contribution No. 931 from the Horticulture Division, Experimental Farms Service, Canada Department of Agriculture, Ottawa, Ont.
[2] Krum Perlite. (Buffalo Perlite Corp., Buffalo, N.Y.).
[3] Jiffy Pots. (Harris Seeds, Rochester, N.Y.)

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—T. B. HARRISON,
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Harrow, Ontario

March 26, 1958

FIGURE 1. Vegetative propagation of softwood cuttings of peach under mist with the aid of perlite in peat containers.

A. Peach cuttings in mist tunnel.

B. Firm softwood cuttings before (2) and after (3 and 4) three weeks in mist chamber.

C. View of Yunnan cuttings in outdoor nursery on July 17, 1957. (Cuttings struck in perlite on May 30, 1957, transferred to nursery June 28, 1957).